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THE BULLETIN

OF THE

NORTH CAROLINA DEPARTMENT OF AGRICULTURE

RALEIGH

Vol. 41, No. 17 DECEMBER, 1920 Whole No. 277

MINERAL CONTENT OF SOUTHERN POULTRY FEEDS

and their

POTENTIAL ACIDITY AND POTENTIAL ALKALINITY

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Mineral Content of Southern Poultry Feeds and of Other Feed Mixtures *and their* Potential Acidity and Potential Alkalinity

By B. F. Kaupp, Poultry Investigator and Pathologist,
Animal Industry Division

INTRODUCTION

The mineral substances which enter into the composition of fowls constitute the ash. These ash constituents stand in a peculiar and interesting relation to the living structures and the life processes of animals. Through experimentation, the physiologist has determined the effects of many of the minerals upon function and secretion which will be briefly reviewed under the proper heading. The ash constituents are also essential to the construction of the body tissues of the fowl. In this field the soil chemist, the agronomist, the poultryman, the physiologist, and the farmer all find a common ground of interest, as these mineral substances required by fowls come from the soil through the plant to the bird.

The force feeding of fowls for growth into broilers, as well as for egg production, as applies to mature hens, calls for a higher percentage of mineral nutrients in feed stuffs than was necessary under the old system of less intense production. Since it is so essential that fowls be supplied with sufficient amounts of each mineral element, studies should be conducted to determine if any of the poultry feeds are deficient and, if they are deficient, what is essential to make good this deficiency. It may not be possible to exercise a selective choice of feeds which provide an abundance of the various minerals for rapidity or efficiency in the production of growth, or eggs.

This publication deals with broiler production alone.

USE OF MINERALS IN THE BODY

The importance of the mineral salts in the vital processes of the animal cell lies almost entirely in their physical or physico-chemical properties. The chemical reactions in the body, which constitute the physical basis of life, take place between substances in solution. It is by means of the electrical charges carried by the particles in solution that reactions are brought about.

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Very slight amounts of the mineral salts, acid or alkalies, in water enormously increase its power to conduct electrical currents. Many of the organic compounds of cells do not possess this capacity and hence show less pronounced chemical properties. Their molecules are usually vastly larger and more complex than those of the inorganic compounds, and the speed with which they carry electrical charges is correspondingly slow, with such as possess this property at all.

Most of the mineral elements in the body are strongly acid, or basic, and their compounds have a tendency to become exceedingly active when in dilute solution; also the small size of the molecules of their simpler compounds allows them to pass freely through cell membranes that are impenetrable to many of the larger molecules of the complex compounds. Forbes[1] says "The mineral substances of animal tissues exist not merely in solution but partly in firm combination with the organic constituents. These mineral substances render chemically active the large and inert organic complexes to which they are bound" and these facts led Mann to say "So-called pure ash-free proteids are chemically inert and in the true sense of the word, dead bodies."[2] Life is put into them by the presence of electrolites or bodies capable of carrying electric charges.

Kinds of Minerals: The minerals of the body occur in the form of calcium oxide, inorganic phosphates, lecithins, phosphoproteins. In the inorganic phosphates, phosphorus is present as salts of the mineral bases, calcium, magnesium, potassium and iron. Sodium occurs principally as sodium chloride and to a less extent as sodium phosphate and sodium carbonate. Potassium is present mostly as salts of mineral acids. Magnesium usually combines with phosphate, forming magnesium phosphate. Sulphur is in combination with proteid bodies. Chlorine is in combination with sodium chloride. Iron is in combination with hemaglobin.

In the body, the base forming elements are calcium, magnesium, sodium and potassium. The acid forming elements are phosphorus, chlorine, and sulphur.

Acid mineral elements enter the body in organic combination as follows: sulphur as a constituent of food, protein. If sulphur is oxidized it is burned into sulphuric acid and excreted in the urine as inorganic sulphates[3]. Sulphates and phosphates result from proteid tissue waste and by oxidation of food protein in the body. These are eliminated from the body as such. Phosphorus enters the body as inorganic phosphates; as salts of various organic acids; as lecithins (compounds of fat) phosphoric acid, and in phosphoproteins and nucleoproteins. The eliminated phosphorus by way of the urine is in the form of di- and mono-hydrogen phosphates of sodium and potassium, and less abundantly in the form of phosphates of calcium and magnesium. The phosphorus of the feces is largely in the form of phosphates. Chlorine enters the body as chlorides and is eliminated by the kidneys almost wholly as chlorides.

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[1] Forbes, E. B., Ohio Agricultural Experiment Station. Bulletin 201, p. 137.
[2] Mann: Chemistry of the proteids, p. 219.
[3] Forbes, E. B., Ohio Experiment Station. Bulletin 207, p. 24.

To summarize, the mineral bases are calcium, sodium, magnesium, and potassium, which enter the body as salts of various organic and inorganic acids. These alkali reacting elements are used in the body mostly in combination with phosphoric, sulphuric, hydrochloric, and carbonic acids, principally for structural, regulative and catalytic purposes, and are eliminated from the body as inorganic salts.

Forbes[4] summarizes as follows: "The organic acids of feedstuffs, such as citric, malic, and tartanic acids of fruits, are mostly oxidized in the animal body to carbondioxide and water, in which compounds they are excreted from the body. There are formed within the body mineral acids which cannot be eliminated in this way. These acids must be neutralized in order to protect the animal from a disturbance of conditions essential to the continuance of vital reactions."

These acids are formed chiefly by the cleavage and oxidation of proteids, either of the body or of the feed, the sulphur and phosphorus contained therein, as constituent parts, being oxidized to the corresponding inorganic acids.

These acids are neutralized:

First—By carbonates of the feed, water, or tissues.

Second—By alkalies liberated by the oxidation of organic-acid salts.

Third—By ammonia withdrawn from constructive formation or urea.

Fourth—By ammonia from the tissues.

Fifth—By ammonia split off from proteids, especially, for acid neutralization.

The practical bearing of the subject is on feeding of such animals as poultry which are largely raised on cereals or their by-products.

There is especially considered in this connection the acid mineral elements, sulphur, phosphorus, and chlorine, and the basic mineral elements, sodium, potassium, magnesium, and calcium.

The relative amounts of mineral acids formed in the body may be greatly modified by a choice of feedstuffs. Alkaline ash is contained in fruits, vegetables, milk, oyster shell and lime stone grit, while acid ash is contained in meat scrap, or meat meal, eggs, cereals, and their by-products.

The bases are more deficient than the acids. The basic mineral elements in a ration must be in quantities corresponding to the protein, since the sulphur and phosphorus of the feed proteins constitute the principal sources of mineral acids in the body. The excess of mineral bases in the feed must be maintained at a high level.

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[4] Forbes, E. B., Ohio Experiment Station. Bulletin 207, p. 49.

In practice, animals do not experience injurious effects from an excess of alkali as they do from an excess of acid. A needless amount of protein unnecessarily taxes the acid-neutralizing capacity of the animal, and if carried to a sufficient extreme, results either in interference of the formation of bone or in its malnutrition. A high fat-content of milk or indigestible character of this fat when fed to individuals suffering from digestive disturbances causes acid intoxication by withdrawal of alkalies, by way of the feces, in the condition of difficult soluble calcium soaps.

It is a known fact that poverty of the soil, or in drought, may affect the ash content of feeds. If the soil is poor in calcium and phosphorus, the crop will be correspondingly poor. Such feeds will limit the growth and development of bone. If a sufficient amount of vegetables, fruits, and milk is given, there is not likely to be an excess of mineral acids above the capacity of the body to neutralize. Clover and alfalfa are specially rich in calcium and, hence, are excellent, either as pasturage, cut green, or ground and fed in the mash, to make good deficiencies in such grains as corn.

Aid to Construction of Tissue: Bone contains calcium in the form of calcium phosphate, carbonate, and fluoride. It contains magnesium in the form of phosphate of magnesium. Sodium chloride is also present.

The fluids of the body contain approximately 0.85 per cent sodium chloride.

Proteid bodies, feathers, and horn contain sulphur.

The hemaglobin of the erythrocyte contains iron. The nucleus of the leucocyte contains phosphorus, as does also prothrombin. The blood also contains considerable amounts of potassium chloride, and sodium carbonate. Phosphates of calcium, magnesium, and sodium are next in abundance; the chief inorganic constituents of the cells being potassium phosphate. In the plasma, sodium chloride is the most abundant salt.[5]

In the construction of the tissues of the growing chick, calcium, phosphorus, potassium, sodium and chlorine play the most important part.

Aid to Function: The presence of calcium salts is necessary for muscular contraction, and the control of both voluntary and involuntary muscles is accompanied through the proportion of calcium, magnesium, sodium, and potassium salts acting upon them. Sodium salts are essential to cardiac relaxation, in fact the heart cannot functionate without the presence of sodium and calcium salts. The blood cannot coagulate, when drawn, without the presence of calcium salts. The essential alkalinity of the blood is due to the phosphate and bicarbonate of sodium. Iron is essential in the red blood corpuscle to give it its oxygen carrying power, 0.4 per cent of the hemaglobin being iron. The nature of the inner stimulus of the heart is intimately connected with certain organic salts of sodium, calcium, and potassium. These are probably in the form of chlorides. It has been shown that calcium promotes contraction, and that sodium and potassium bring about relaxation of the heart. The sodium carbonate of the blood probably assists in carrying the carbon di-oxide to the eliminative organs, the lungs. Bohr[6] disputes this point and maintains that the carbon di-oxide is carried in loose chemical union with the hemaglobin.

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[5] Smith, F., A Manual of Veterinary Physiology. Third Edition.

Free acid formation in the stomach of fowls is probably formed by selective powers, possessed by the secretive cells, by an interaction of sodium chloride and sodium di-hydrogen phosphate of the blood. The digestive fluid secreted by the glands of Lieberkuhn is alkaline due to sodium carbonate. The pancreatic secretion contains much sodium, magnesium, potassium, and calcium in combination with chloride, carbonate, and phosphate. Bile contains sulphur, phosphate and chloride of sodium, and salts of calcium, magnesium, iron, and potassium. The larger proportion of salt is sodium.

The salts of the body perform important functions in connection with secretion and excretion. They direct the metabolism of the body, though how is little understood. They regulate the fluid flow from blood to tissues and vice versa. Irritability of muscle and nerve is due to salts.

Young growing birds require more salts than adults. The activity of enzymes, whose function in building up and tearing down chemical compounds in the animal body, requires certain degrees of acidity and alkalinity is maintained in proper degree through the presence of mineral salts. Animals can live but a short time on feeds free from mineral salts[7]. Forster[8] fed pigeons on practically ash free feed. They died in a few days. He considered that the lack of mineral matter was the cause of death. Alkali bases furnished by the liver through its bile are necessary to fat digestion.

Salts assist in the formation of secretions, repair and disintegration. The growth of the solid tissues depends on the inorganic material supplied by the blood. Water free from salts is destructive to protoplasm. One important function of the salts in the blood is to maintain the vitality of the tissues. This is one of the principal functions of sodium chloride.

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[6] Ibid.
[7] Forster, J.:I Zlscter. f. Biol. Vol. IX, p. 297.
[8] Ibid.

The excess of mineral bases in the food must be maintained at a high level.

FATE OF ADDITIONAL MINERALS GIVEN IN FEED

Hunt[9] of the Laboratory of Experimental Pharmacology and Therapeutics of the Harvard Medical School gives the following as his opinion of the fate of some of the salts added to feed mixtures: "It is my impression that only a small amount of the calcium and magnesium, in the formula you propose, would be absorbed; that the potassium chloride would be completely and quickly absorbed and, unless the body needed it, would be equally quickly excreted by the kidneys. At least some sodium sulphate is absorbed; probably much of it if the quantity is small. The sodium of the NaOH would certainly be absorbed."

Wells[10], Director of Medical Research of the University of Chicago, gives the following as his opinion: "The character of the compounds that will form in a mixture such as you describe will depend entirely upon the concentration of the different ingredients. In the case of the carbonates with volatile anions you would naturally get complete substitution by the chlorine or lactic acid radical and therefore have a mixture of sodium lactate and sodium chloride or the corresponding calcium salts, in the presence of sour milk. With the sodium sulphate and potassium sulphate the amount of formation of sodium chloride or sodium lactate will be entirely a proposition of mass action and the sulphates and chlorides will predominate. In any case, all the compounds that would be formed would be entirely absorbed. It is always hard to estimate how much calcium salts is absorbed because a considerable portion of this is always secreted back into the intestines and no matter how much is given by the mouth of any calcium salt but a small amount passes out in the urine and can be found circulating in the blood at any one time."

The salts are of no value from an energy standpoint. Their changes are not accompanied by the liberation of heat energy, or if so, this is so small that it has never been measured[11]. They do, however, maintain a normal composition and osmotic pressure. They play an important part in controlling the flow of water to and from the tissues. They constitute the essential part of living tissues, and are essential to its normal reactions.

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[9] Private correspondence.
[10] Private correspondence.
[11] Smith, F. A., Manual of Veterinary Physiology. Third edition.

In giving minerals in the feed, the changes that follow in the stomach and intestines are of interest.

Magnesium carbonate is an antiacid and neutralizes abnormal acidity, due to fermentation when exhibited after meals. It unites with the gastric juice to form chlorides, lactates, and bicarbonates. The acidity of the milk may also be partially neutralized. These compounds are milk saline purgatives. The carbonate of magnesium renders alkaline the blood and urine, and is a slight diuretic. It resembles potassium and sodium bicarbonate, as antiacids, but is milder because feebly absorbed.

Phosphorus, when given in small doses to growing animals, apparently stimulates the bone making cells, or osteoblasts, and the growth of denser bone, both from cartilage and periosteum. In fowl, the marrow cavity may be wholly obliterated by the deposition of hard bone through the ingestion of phosphorus. Phosphorus is apparently a nerve stimulant. Phosphorus is absorbed largely in an unchanged condition, in solution, in fatty matter, in the bowels, and as vapor. Some of the phosphorus is probably converted in the bowels and blood into phosphoreted hydrogen (PH_3), and further oxidized into phosphoric acid in the body. The fate is unknown but some phosphorus is eliminated as vapor from the lungs, and some inorganic compounds in the urine. Vegetables supply much potassium salts.

Calcium salts mostly pass unchanged through the bowels. A small amount is absorbed and eliminated by the large bowel and by the kidneys. Calcium chloride causes the blood to coagulate more quickly and, for this purpose, has been used therapeutically. Calcium carbonate is one of the slowest acting antiacids, because of its comparative insolubility. It is excreted unchanged in the feces.

Sodium chloride is constantly being eliminated by the urine. According to Bunge, potassium salts on entering the blood bring about a chemical reaction, whereby the sodium chloride circulating in the plasma is split; the chloride in sodium chloride combines with potassium, while the acid set free from the potassium salt unites with sodium and both products are swept away with the urine, thus removing sodium chloride from the blood. This must be replaced by that taken in by the feed. Animals deprived of sufficient sodium chloride suffer from anemia, general weakness and edema. Large doses causes irritation of the stomach and bowels. Sodium chloride augments the red blood corpuscles. Salt solutions in the blood withdraw fluid lymph from the tissues by osmotic action. In this way they appear to stimulate change, as there is an increased elimination of nitrogen in the urine. Vascular tension is augmented and activity of the Malpighian bodies, and therefore diuresis.

Sodium sulphate is a cathartic and is readily absorbed into the blood. It also causes diuresis.

Potassium chlorate in concentrated form is an irritant. It does not appear that we have here any compound which is from 0.5 to 1.5 per cent should be caustic or excessively irritant.

The action of salts on the body is determined, not by the action of chemical compounds or its molecule, but by the action of the ions which are dissociated when the salt goes into solution.

OBJECTS OF THE EXPERIMENT

It can be seen from the foregoing that the proper kinds and quantities of mineral elements are essential to the proper well being of the bird. These are essential for the proper construction of new tissue and of eggs in laying hens; for proper functioning of the various organs of the body; for an aid in the transfer of both useful compounds to the various tissues and cells of the body and for the taking away of the unuseful or waste products.

For these reasons it is essential to know just what mineral elements are essential and in what quantities they are needed for the purpose for which the bird is being fed; that is, whether for mature hens for egg production, for broilers for growth and development, or for fattening fowls.

These problems in their different phases will require much study. This bulletin discusses only one phase, that of medium broiler production—the raising of the chick from hatching to eight weeks of age, at which time the flock averages should be one and one-half pounds each.

OUTLINE OF EXPERIMENT

As stated, the object of this line of experimental work is to determine the amount of mineral required in broiler production. Profitable broiler production begins with the baby chick and extends over a period of about eight weeks, at the end of which time the birds should weigh, as a flock average, approximately one and one-half pounds each.

The first object was to determine the amount of mineral per unit in the bodies of the baby chick, and of the one and one-half pound broiler; to determine the mineral content of the southern poultry feeds; to prepare proper feed mixtures from the standpoint of protein, carbohydrate, and fat; to determine the mineral content, and finally determine if the mineral in the feed mixtures was in sufficient quantities for the greatest possible rate of growth.

The baby chicks were produced from a single flock of pure bred Single Comb White Leghorns, bred on the Station and College Poultry

Plant, and were hatched in one electrobator and each lot housed under similar conditions using similar oil burning hovers of 100-chick capacity.

A room with concrete floor, with plenty of light and ventilation, was used in which to raise the flock. The birds were confined to runs six feet square with smooth galvanized iron for the floor. The hover was set in the center of this square. The work was to be carried on in periods of seven days each and extending over at least eight periods or weeks. At the end of each period the chicks were removed for weighing and cleaning the runs. All material which adhered to the feet was carefully removed, the floor swept and then scraped, being careful not to scrape off any of the metal. The metal floor then made washing possible. This was done with distilled water and by the aid of a brush. This material was drained into a pan and the material placed in an evaporating oven and the moisture driven off. There is a possibility that some errors may have crept in in these processes, but at every turn our whole thought was to be very careful and accurate. Young vigorous chicks are very active and we believe that it would be a miracle indeed to carry on practical flock experiments without slight errors at the most.

Young chicks have a habit of throwing out their feed from the feed box with their beaks, and, especially if they are not particularly hungry and simply looking for something that might be very palatable. In order to avoid this wastage, we constructed double boxes shown in the accompanying illustration. The clabbered skim milk was given in 50 c.c. beakers, and these were set in the ends of the boxes so that they could not be turned over and thus lose the milk and injure our records. The chick-size limestone grit and the chick-size oyster shell were given in petri dishes, and these placed in another container (see c of illustration). The dry mash, grain mixture, and the cut green feed, was placed in double boxes, b, d, and e, as illustrated in the accompanying cut. Figure f of the illustration shows the rape used in the experiments. The feed thrown out of these double boxes was caught in the outside chamber, and was thus easily recovered. The droppings which were deposited in the boxes, after drying, were easily removed by aid of a pair of forceps. These birds received no water, the liquid intake being milk.

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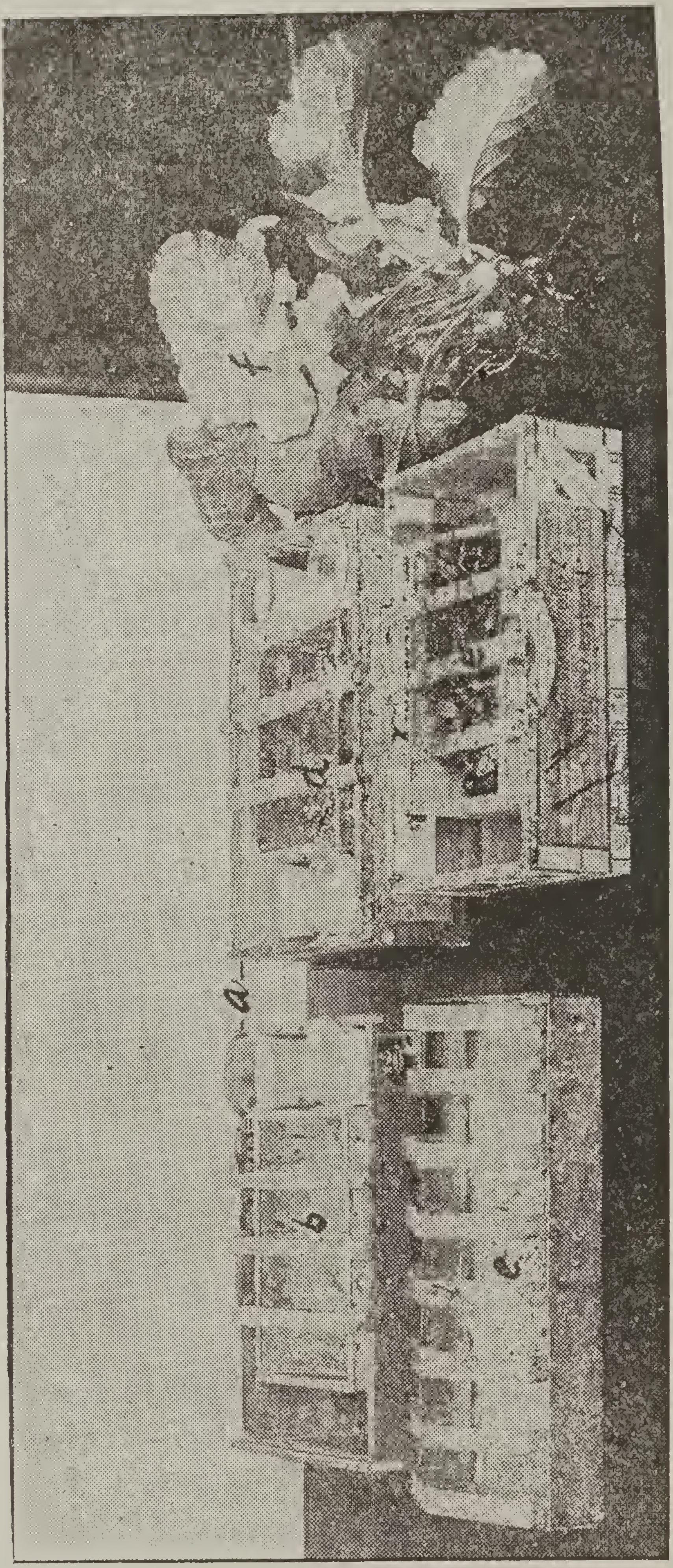
a. milk containers; b. mash; c. grit and shell; d. grain; c. green cut feed; f. rape.



A. Milk Containers; B. Mash; C. Grit and Shell; D. Grain; E. Green Cut Feed; F. Rape

TABLE I

MINERAL CONTENT OF BODIES OF FOWLS*

Age	Parts per Hundred							
	K	Na	Ca	Mg	S	Cl	P	Fe
Baby chick *a*	0.2922	0.2774	0.1978	0.0028	0.0 07	0.1510	0.355	0.0054
1½ lb. broiler *b*	.2380	.1580	1.0340	.0440	.3030	.0790	1.288	.0056
1 year old hen *c*	.2750	.1640	1.2970	.0510	.3820	.2080	1.510	.0066

*All tables of analyses in this bulletin were made by Dan M. McCarty, Nutritional Chemist, Animal Industry Division, N. C. Experiment Station, and Jno. E. Ivey of this laboratory.

a The baby chicks were taken from the incubator, killed with chloroform, the abdominal yolk sacs removed.

b The analyses was of a Single Comb White Leghorn.

c The one year old hen was a Columbian Wyandotte.

TABLE II

MINERAL CONTENT OF SOUTHERN POULTRY FEEDS*

(Results expressed in parts per hundred)

Feed	No. of Analyses	Potassium	Sodium	Calcium	Magnesium	Sulphur	Chlorine	Phosphorus	Iron
Corn meal, bolted	4	0.349	0.072	0.0092	0.1336	0.160	0.0244	0.341	0.0040
Pinhead oats	7	0.441	0.109	0.0126	0.0704	0.236	0.0900	0.490	0.0019
Whole wheat	13	0.435	0.039	0.0271	0.1127	0.183	0.0630	0.436	0.0070
Wheat middlings	6	0.949	1.219	0.0980	0.3628	0.232	0.0603	0.783	0.0052
Bone meal	3	0.229	0.735	21.1770	0.5800	0.170	0.0900	10.349	0.0180
Meat and bone meal	8	0.158	0.473	13.2927	0.8333	0.238	0.5883	5.029	0.1252
Rolled oats	2	0.370	0.136	0.0430	0.1560	0.256	0.0238	0.473	0.0062
Whole corn	13	0.332	0.041	0.0127	0.1051	0.148	0.0521	0.293	0.0044
Hulled oats	6	0.387	0.053	0.0915	0.1465	0.204	0.0870	0.454	0.1090
Velvet bean meal†	1	1.186	0.141	0.3600	0.2080	0.151	0.2220	0.764	0.0126
Soybean meal	5	1.179	0.422	0.2901	0.2503	0.330	0.0373	0.481	0.0241
Peanut meal	5	1.191	0.422	0.2401	0.2106	0.325	0.0312	0.567	0.0271
Skim-milk	2	0.151	0.144	0.1530	0.0019	0.042	0.0650	0.136	0.0036
Egg, inc. shell	3	0.0103	0.200	0.6080	0.0985	0.395	0.1500	0.3021	0.0103
Rape, green	3	0.2510	0.008	0.0084	0.0206	0.035	0.0930	0.0102	0.00000076
Lime stone grit	2	0.0000	0.000	30.9700	6.6700	0.000	0.0000	0.0000	3.3330
Oyster shell	2	0.0000	0.000	37.9510	0.4200	0.147	0.0900	0.0000	0.37500
Dried buttermilk	4	0.7084	0.9531	1.3491	0.8107	0.084	0.3650	0.1691	0.4301
Digester tankage	4	0.3605	0.1948	3.0147	1.1654	0.124	0.3483	1.2323	0.10175
Dried blood	4	0.0941	0.3223	0.3755	0.2175	0.281	0.1381	0.2864	0.01650

*Average of all our analysis to March 1, 1920

†Velvet bean meal and pod.

In making up the feed mixtures for these experiments, each kind of feed was taken from a single sack.

TABLE III

The analyses of the feed in each sack is given in the following table:

Feed	Parts per Hundred							
	K	Na	Ca	Mg	S	Cl	P	Fe
1a—Corn, bolted	0.265	0.076	0.0102	0.1318	0.163	.0232	0.3310	0.0018
1b—Pinhead oats	.467	.115	.0100	.0557	.264	.1050	.5190	.0020
1c—Rolled oats	.370	.149	.0430	.1557	.276	.0249	.4620	.0072
4d—Cracked wheat	.437	.034	.0240	.1027	.182	.0650	.4840	.0022
1e—Cracked corn	.344	.038	.0170	.0667	.195	.0550	.3012	.0028
1f—Wheat middlings	.694	.646	.0840	.3728	.238	.1050	.9420	.0048
1g—Bone meal	.145	.886	22.0550	.5700	.180	.070	10.3690	.0150
1h—Meat and bone meal	.179	.304	13.3330	.2700	.350	.8200	6.5700	.0500
1i—Hulled oats	.374	.084	.056	.1542	.235	.060	.4060	.0030
1j—Limestone grit	.000	.000	30.970	6.6700	.000	.000	.0000	3.3300
1k—Oyster shell	.000	.000	37.951	.4200	.147	.090	.0000	.3750
1m—Skim-milk	.151	.144	.153	.0018	.0424	.0650	.1360	.0036

THE FEED MIXTURES

The following are the feed mixtures used in these experiments:

MIXTURE 1

1c—Rolled oats, 8 parts*.
1f—Wheat middlings, 8 parts.
1h—Meat and bone meal, 2 parts.
1g—Bone meal, 1 part.

MIXTURE 2

4d—Cracked wheat, 3 parts.
1e—Cracked corn, 2 parts.
1b—Pinhead oats, 1 part.

MIXTURE 3

1f—Wheat middlings, 6 parts.
1a—Corn meal, 3 parts.
1h—Meat and bone meal, 3 parts.
1g—Bone meal, 1 part.

MIXTURE 4

4d—Whole wheat, 3 parts.
1e—Cracked corn, 2 parts.
1i—Hulled oats, 1 part.

These chicks were given all the mash and skim milk they would consume. They were Single Comb White Leghorns from pen 14 of the Station and College flock, were hatched in the same electrobator, and were brooded under similar oil burning 100-chick size hovers. Thus all factors are as nearly the same as could be secured.

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*Parts by weights.

Much of the detailed tables will be omitted from this bulletin, giving only final tables.

TABLE IV

FEED AND MINERAL INTAKE LOT 3

First Period

Feed Milk	Amount Grams	K	Na	Ca	Mg	S	Cl	P	Fe
Feed milk	1510	2.280	2.174	2.310	0.0271	0.6402	0.9815	2.0536	0.0543
M No. 1	299	1.417	1.235	7.826	.8398	.7842	.4326	5.4660	.0328
M No. 2	45	.184	.027	.008	.0369	.0900	.0292	.1930	.0010
Grit	76	.000	.000	23.537	5.0692	.0000	.0000	.0000	2.5308
Totals	1930	3.881	3.436	33.678	5.9730	1.5144	1.4433	7.7126	2.6189

Second Period

Feed milk	2492	3.762	3.588	3.812	0.0448	1.0566	1.6198	3.3891	0.0897
M No. 1	391	1.854	1.616	10.234	1.0983	1.0255	.5657	7.1478	.0430
M No. 2	275	1.124	.132	.052	.2255	.5500	.1787	1.1794	.0063
Rape	156	.391	.012	.013	.0321	.0552	.1450	.1600	trace
Totals	3314	7.131	5.348	14.111	1.4007	2.6873	2.5092	11.8763	.1390

Third Period

Milk	2356	3.557	3.392	3.604	0.0424	0.9989	1.5314	3.2041	0.0848
M No. 1	499	2.366	2.062	13.062	1.4016	1.3088	.7220	9.1222	.0099
M No. 2	368	1.505	.176	.070	.3017	.7360	.2392	1.5783	.0084
Rape	385	.966	.030	.032	.0793	.1362	.3580	.3950	trace
Grit	22	.000	.000	6.813	1.4674	.0000	.0000	.0000	.7326
Oyster shell	11	.000	.000	4.174	.0462	.0161	.0099	.0000	.0412
Totals	3641	8.394	5.660	27.755	3.3386	3.1960	2.8605	14.2996	.8769

Fourth Period

Milk	2545	3.842	3.664	3.893	0.0458	1.0790	1.6542	3.4612	0.0916
M No. 2	547	2.237	.262	.105	.4485	1.0940	.3555	2.3460	.0125
M No. 3	523	2.264	2.369	25.179	1.6108	1.2656	1.2980	14.7737	.0800
Rape	342	.858	.027	.028	.0704	.1210	.3180	.5308	trace
Grit	8	.000	.000	2.477	.5336	.0000	.0000	.0000	.2664
Oyster shell	17	.000	.000	6.451	.0714	.0249	.0153	.0000	.0637
Totals	3982	9.201	6.322	38.133	2.7805	3.5845	3.6410	21.1117	.5142

Fifth Period

Feed Milk	Amount Grams	K	Na	Ca	Mg	S	Cl	P	Fe
Milk	3248	4.904	4.677	4.969	0.0584	1.3771	2.1112	4.4172	0.1169
M No. 2	1247	5.100	.598	.239	1.0225	2.4940	.8105	5.3483	.0286
M No. 3	334	1.446	1.513	16.080	1.0287	.8082	.8289	0.4348	.0511
Rape	321	.805	.025	.026	.0661	.1136	.2985	.3293	trace
Grit	18	.000	.000	5.574	1.2006	.0000	.0000	.0000	.5994
Totals	5168	12.255	6.813	26.888	3.3763	4.7929	4.0491	19.5296	.7960

Sixth Period

Feed Milk	3400	5.134	4.896	5.202	0.0612	1.4416	2.2100	4.6240	0.1224
M No. 2	1353	5.533	.649	.259	1.1094	2.7060	.8794	5.8030	.0311
M No. 3	381	1.649	1.725	18.343	1.1734	.9220	.9456	10.7624	.0582
Rape	912	2.289	.072	.076	.1878	.3228	.8481	.9357	trace
Grit	33	.000	.000	10.220	2.2011	.0000	.0000	.0000	1.0989
Totals	6079	14.605	7.342	34.100	4.7329	5.3924	4.8831	22.1251	1.3106

Seventh Period

Milk	3989	6.023	5.744	6.103	0.0718	1.6913	2.5928	5.4250	0.1436
M No. 3	332	1.437	1.503	15.984	1.0225	.8034	.8240	9.3783	.0507
M No. 4	1236	4.869	.531	.333	1.2261	2.4102	.7514	5.0676	.0247
Rape	880	2.208	.070	.073	.1812	.3115	.8184	.9028	trace
Grit	39	.000	.000	12.078	2.6013	0.0000	.0000	.0000	1.2987
Totals	6476	14.537	7.848	34.571	5.1029	5.2164	4.9866	20.7737	1.5177

Eighth Period

Milk	4203	6.346	6.052	6.430	0.0756	1.7820	2.7319	5.7160	0.1513
M No. 3	382	1.654	1.730	18.391	1.1765	.9244	.9481	10.7907	.0584
M No. 4	1484	5.846	.638	.400	1.4721	2.8938	.9022	6.0844	.0296
Rape	731	1.834	.058	.061	.1505	.2587	.6798	.7500	trace
Totals	6800	15.680	8.478	25.282	2.8747	5.8589	5.2620	23.3411	.2393

TABLE V

This table gives the mineral content of the four feed mixtures. Parts per hundred.

Mixture	K	Na	Ca	Mg	S	Cl	P	Fe
1	0.4742	0.4133	2.61764	0.2809	0.2623	0.1447	1.8281	0.0110
2	.4090	.0480	.01920	.0820	.2000	.0650	.4289	.0023
3	.433	.453	4.81446	.3080	.2420	.2482	2.8248	.0153
4	.394	.043	.02699	.0992	.1950	.0608	.4100	.0020

TABLE VI

This table gives the weights of the chicks at hatching and at the end of each of the eight periods.

Number of Chick	Weight at Hatch—Grams	Weight at 7 Days	Weight at 14 Days	Weight at 21 Days	Weight at 28 Days	Weight at 35 Days	Weight at 42 Days	Weight at 49 Days	Weight at 56 Days
902	41	57	91	128	135	205	280	350	460
904	45	57	83	123	165	215	253	265	316
906	41	59	88	132	169	245	311	365	430
910	40	60	98	145	197	245	308	355	420
912	42	58	85	110	168	219	287	335	411
914	40	52	65	113	149	205	278	320	400
918	42	59	75	110	151	203	252	275	329
921	42	70	77	108	154	200	271	335	438
922	36	57	90	137	187	250	300	345	410
924	43	59	90	130	150	185	215	240	282
926	44	57	79*	---------	---------	---------	---------	---------	---------
928	42	45	65*	---------	---------	---------	---------	---------	---------
Totals	498	690	986	1236	1625	2172	2755	3185	3896

TABLE VII

This table gives the per cent increase gain in weight at the end of each 7-day period:

Chick Number	1 P	2 P	3 P	4 P	5 P	6 P	7 P	8 P
902	28	37	28	5	34	26	20	23
904	21	31	32	25	23	15	4	16
906	30	31	33	21	31	21	14	15
910	50	38	32	26	15	20	13	15
912	27	31	22	28	23	24	14	16
914	23	20	42	24	27	26	13	13
918	28	20	31	26	25	19	8	16
921	40	10	28	29	23	26	19	23
922	36	36	34	26	25	16	13	15
924	27	34	30	13	18	13	10	14
926	22	27*	---------	---------	---------	---------	---------	---------
928	6	30*	---------	---------	---------	---------	---------	---------
Flock average	27	30	31	23	25	21	13	18
Total gain in grams	192	296	394	389	547	583	430	711

*Died

TABLE VIII

This table gives the amount of gain in each period, and the amount of mineral elements contained in this amount of tissue, based on the analyses of the bodies of the baby chick.

Period	Grams Gain	K	Na	Ca	Mg	S	Cl	P	Fe
First	192	0.5610	0.5326	0.3797	0.0053	0.0205	0.2899	0.6816	0.0103
Second	296	.8649	.8211	.5854	.0082	.0316	.4469	1.0508	.0159
Third	394	1.1512	1.0929	.7793	.0110	.0421	.5949	1.3987	.0212
Fourth	389	1.1366	1.0790	.7694	.0108	.0416	.5873	1.3809	.0210
Fifth	547	1.5983	1.5173	1.0819	.0153	.0585	.8259	1.9418	.0295
Sixth	583	1.7035	1.6172	1.1531	.0163	.0623	.8803	2.0696	.0314
Seventh	430	1.2564	1.1928	.8505	.0120	.0460	.6493	1.5265	.0232
Eighth	711	2.0775	1.9723	1.4063	.0199	.0760	1.0736	2.5240	.0383

TABLE IX

This table gives the amount of gain in each period, and the amount of mineral elements contained in this amount of tissue, based on the analyses of the one and one-half pound Single Comb White Leghorn broilers.

Period	Grams Gain	K	Na	Ca	Mg	S	Cl	P	Fe
First	192	0.4569	0.3033	1.9852	0.0844	0.5817	0.1516	2.4729	0.0107
Second	296	.7044	.4676	3.0606	.1302	.8968	.2338	3.8124	.0165
Third	394	.9377	.6225	4.0739	.1733	1.1938	.3112	5.0747	.0220
Fourth	389	.9258	.6146	4.0222	.1711	1.1786	.3073	5.0103	.0217
Fifth	547	1.3018	.8642	5.6559	.2406	1.6574	.4321	7.0453	.0306
Sixth	583	1.3875	.9211	6.0282	.2565	1.7664	.4605	7.5090	.0326
Seventh	430	1.0234	.6794	4.4462	.1892	1.3029	.3397	5.5384	.0240
Eighth	711	1.6921	1.1233	7.3517	.3128	2.1543	.5616	9.1576	.0398

TABLE X

This table gives the mineral intake supplied by the feed, the outgo by way of the bowel, the amount of each element required to build up the tissue gain based on the analyses of the bodies of one and one-half pound broilers, and the mineral balance.

Period	K Intake Outgo Required Balance	Na Intake Outgo Required Balance	Ca Intake Outgo Required Balance	Mg Intake Outgo Required Balance	S Intake Outgo Required Balance	Cl Intake Outgo Required Balance	P Intake Outgo Required Balance	Fe Intake Outgo Required Balance
First	3.8810	3.4360	33.6780	5.9730	1.5144	1.4433	7.7126	2.6189
	1.9847	1.0445	16.7695	2.3497	0.6728	0.7905	3.8349	0.0020
	.4569	.3033	1.9852	.0844	.5817	.1516	2.4729	.0107
	+1.4394	+2.0882	+14.9233	+3.5389	+ .2599	+ .5012	+1.4048	+2.6062
Second	7.1310	5.3480	14.1110	1.4007	2.6873	2.5926	11.8763	0.1390
	5.7272	2.2206	15.9993	5.1619	2.3389	2.0876	8.3507	.0203
	.7044	.4676	3.0606	.1302	.8968	.2338	3.8124	.0165
	+ .6994	+2.6598	—4.9489	—3.8914	— .5484	+ .1878	— .2868	+ .1022
Third	8.3940	5.6600	27.7550	3.3386	3.1960	2.8605	14.2996	0.8769
	6.4260	3.0704	17.0000	2.4208	1.3804	3.1790	9.1120	.0340
	.9377	.6225	4.0739	.1733	1.1938	.3112	5.0747	.0220
	+1.0303	+1.9671	+6.6811	+ .7445	+ .6218	— .6297	+ .1129	+ .8209
Fourth	9.2010	6.3220	38.1330	2.7805	3.5845	3.6410	21.1110	.5142
	7.7360	4.5841	30.4734	2.6014	1.8724	2.8585	16.9410	.0314
	.9258	.6146	4.0222	.1711	1.1786	.3073	5.0105	.0217
	+ .5392	+1.1233	+3.6374	+ .0080	+ .5335	+ .4752	— .8405	+ .4611
Fifth	12.2550	6.8130	26.8880	3.3763	4.7929	4.0491	19.5296	.7960
	9.5486	3.7437	32.1895	3.6274	2.6184	3.4894	14.9857	.0324
	1.3018	.8642	5.6559	.2406	1.6574	.4321	7.0453	.0306
	+1.4046	+2.2051	+10.9574	— .4917	+ .5171	+ .1276	—2.5014	+ .7330
Sixth	14.6050	7.3420	34.1000	4.7329	5.3924	4.8831	22.1251	1.3106
	13.1084	5.9358	31.8477	5.0382	3.0062	3.4293	18.0121	.0448
	1.3875	.9211	6.0282	.2565	1.7664	.4605	7.5090	.0326
	+ .1091	+ .4851	—3.7759	— .5618	+ .6198	+ .9933	—3.3960	+1.2332
Seventh	14.5370	7.8480	34.5710	5.1029	5.2164	4.9866	20.7737	1.5177
	13.3769	4.4705	21.7299	3.3716	3.3460	3.8460	16.6019	.0233
	1.0234	.6794	4.4462	.1892	1.3029	.3397	5.5384	.0240
	+ .1367	+2.6981	+8.3949	+1.5421	+ .5675	+ .8009	—1.3666	+1.4704
Eighth	15.6800	8.4780	25.2820	2.8747	5.8589	5.2620	23.3411	0.2393
	16.5110	5.9803	33.2590	5.9882	4.0527	4.5030	19.2760	.0395
	1.6921	1.1233	7.3517	.3128	2.1543	.5616	9.1576	.0398
	—2.5231	+1.3744	—15.3287	—3.4263	— .3481	+ .1974	—5.1576	+ .1600
Balance 8 periods	+2.8356	+14.6011	+20.5406	—2.5371	+2.2231	+2.6542	—12.0312	+7.5870

TABLE XI

This table gives the total amount of mineral in the droppings for each period.

Period	K	Na	Ca	Mg	Fe	P	S	Cl	Total Droppings Grams
First	1.9847	1.0445	16.7695	2.3497	0.0020	3.8349	0.6728	0.7905	168.2
Second	5.7272	2.2206	15.9995	5.1619	.0203	8.3507	2.3389	2.0876	369.5
Third	6.4260	3.0704	17.0000	2.4208	.0340	9.1120	1.3804	3.1790	340.
Fourth	7.7363	4.5841	30.4734	2.6014	.0314	16.941	1.8724	2.8585	524.5
Fifth	9.5486	3.7437	32.1895	3.6274	.0324	14.9857	2.6184	3.4894	541.
Sixth	13.1084	5.9358	31.8477	5.0382	.0448	18.0121	3.0062	3.4293	641.
Seventh	13.3769	4.4705	21.7299	3.3716	.0233	16.6019	3.3460	3.8460	641.
Eighth	16.5110	5.9803	33.2590	5.9882	.0395	19.2760	4.0527	4.5030	790.

DISCUSSION

In a study of the mineral content of poultry feeds, it is noted that the different samples from products grown in different sections of the country, or even different sections of the State, and on different plots of ground, vary in their inorganic content. Thus, an average of a large number of analyses from different lots of the same kind of feeds has been given to show the average of these specific analyses, and later a tabulation of the analyses of just the feeds used in these experiments. The latter table will enable one to definitely determine just the amount of mineral taken in during these feeding experiments under discussion. Not only may poverty of the soil affect the mineral content of the feed, but also seasonal variations from year to year, such as drought.

In the baby chick the bones are very thin walled and bend easily, rather indicating that thorough calcification has not taken place in all parts. It is expected that later in the chick's life there would be a greater amount of mineral in the bones, and, consequently, a greater percentage in the total weight of the bird. If the results of the analyses which were carried on with the bodies of baby chicks, and of one and one-half pound broilers are studied, it will be noted that there is a material increase of the greater essential inorganic constituents of the bone, namely—calcium, magnesium, and phosphorus. The baby chick is provided with down which has gradually been replaced with a coat of feathers as the chick develops, and this calls for an increase of sulphur. It is noted also that in this element there has been a material increase. If it is desired to carry out this comparative study a step further, as the bird develops to maturity, it can be seen that there is a still greater increase in calcium, magnesium, phosphorus, and sulphur. The iron content is only slightly increased as the bird develops to maturity.

The bird has no sweat gland and only one oil gland, the latter a double lobulated tubular gland located dorsally at the base of the tail, and which gland furnishes oil for the bird to distribute to each feather by aid of its beak. The excretions from the body of the fowl are by way of the lungs, kidneys, and intestinal tract. The ureters, large intestine, oviduct, and vassa deferentia, all empty into a reservoir, an expansion of the terminal end of the gut, called the cloaca which, in turn empties through the anus to the external world. This arrangement makes the isolation of elements eliminated by the kidneys a difficult task, in fact impossible except through surgical interference, and this has many difficulties. In these particular experiments here discussed this has not been attempted.

In an average of two lots in this series of experiments, the following amounts of feeds were required to produce one gram of gain in weight: milk, 7.49; mash and grain mixtures, 2.91; green feed, 1.00; total, 11.44 grams. In these cases the feed was kept constantly before the flocks, so that the amount of consumption was a maximum amount and by selection, so far as the milk, mash, and grain mixtures were concerned. The rape, finely chopped, and the milk were likewise kept in separate containers. Thus, where Single Comb White Leghorn chicks in their first eight weeks are given all the skim milk and green feed they will consume, there will be required approximately 3 grams grain and mash per gram gain, or in practical terms 3 pounds mash and grain for each pound gain in body weight. In these two lots 75.2 per cent of the carbohydrates was digested and 80.2 per cent of the fat digested. These are averages for the eight periods, the digestibility varying from period to period. We do not believe the methods used to separate the ammonia and uric acid of the feces from the undigested protein of the feed sufficiently accurate to give here. The problem of separating the mineral elements, from those passed out with the feces unused, is quite a different matter. If it were possible by surgical interference to separate the urine from the feces, there would yet remain that eliminated by way of the bowel, which could not be separated from that taken with the food and not utilized. At this time only one practical way is seen to measure mineral requirements, which is by comparing the intake with the outgo and the amount contained in the new constructed tissue, and study the mineral balances left over unaccounted for.

From the table, giving the mineral contents of the bodies of fowls, can be seen the requirements in utilizable mineral to construct a given gain. From the above estimate of the quantity of feeds to produce a pound of gain can be estimated the amount of mineral elements contained in the feed.

The mineral intake will fluctuate with the kinds of minerals given in addition to the dry mash and the grain mixtures. By a study of the table of average analyses we note that milk contains quite a quantity of phosphorus, calcium, sodium, and potassium. The intake of sour skim milk, if given what they will consume, amounts to much in the way of mineral elements. Thus in the first period 58 per cent of the potassium, 63 per cent sodium, 6 per cent calcium, .0045 per cent magnesium*, 42 per cent sulphur, 68 per cent chlorine, 26 per cent phosphorus, and 2 per cent iron* was furnished by the sour skim milk.

In the second period 52 per cent of the potassium, 67 per cent sodium, 27 per cent calcium, 3 per cent magnesium, 39 per cent sulphur, 64 per cent chlorine, 28 per cent phosphorus, and 64 per cent iron was furnished by the sour skim milk.

In the third period 42 per cent of the potassium, 60 per cent sodium, 13 per cent calcium, 1 per cent magnesium, 31 per cent sulphur, 53 per cent chlorine, 22 per cent phosphorus, and 9 per cent iron was furnished by the milk.

In the fourth period 41 per cent of the potassium, 57 per cent sodium, 10 per cent calcium, 1 per cent magnesium, 30 per cent sulphur, 45 per cent chlorine, 16 per cent phosphorus, and 17 per cent of the iron was furnished by the skim milk.

In the fifth period 40 per cent of the potassium, 68 per cent sodium, 18 per cent calcium, 1 per cent magnesium, 28 per cent sulphur, 52 per cent chlorine, 22 per cent phosphorus, and 14 per cent iron was furnisked by the skim milk.

In the sixth period 35 per cent of the potassium, 66 per cent sodium, 15 per cent calcium, 1 per cent magnesium, 26 per cent sulphur, 45 per cent chlorine, 20 per cent phosphorus, and 9 per cent iron was furnished by the sour skim milk.

In the seventh period 41 per cent of the potassium, 73 per cent sodium, 17 per cent calcium, 1 per cent magnesium, 32 per cent sulphur, 52 per cent chlorine, 26 per cent phosphorus, and 9 per cent iron was supplied by the sour skim milk.

In the eighth period 40 per cent of the potassium, 70 per cent sodium, 25 per cent calcium, 2 per cent magnesium, 32 per cent sulphur, 51 per cent chlorine, 24 per cent phosphorus, and 63 per cent iron was supplied by the sour skim milk.

Considering the total mineral nutrients that would have been supplied by the feed ingested, leaving out the milk, there would have been ample furnished in the first, second, third, fourth, fifth, sixth, seventh, and eighth periods.

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*It must be remembered that all the mineral elements in the grit are not liberated for use in the same period in which it is consumed, since the grit will not all be ground for several weeks.

If the birds had not received milk or mash, and estimating that the same quantity of feed consumed as grain mixture and no mash, and not taking into account the consumption of shell or limestone as grit, there would have been in the first period a deficiency in all mineral elements except potassium and magnesium. In the second period there would have been a deficiency in sodium, calcium, potassium, and iron. In the third period there would have been a deficiency in sodium, calcium, phosphorus, and iron. In the fourth period there would have been the same deficiency as in the third period. In the fifth period there would have been a deficiency in calcium alone. There would also have been a deficiency in calcium in the sixth and seventh periods and a deficiency in calcium, sodium, and phosphorus in the eighth period.

In the table giving the mineral balances for the eight periods, there is an apparent balance of 20 grams of calcium unaccounted for, and in this connection it must be remembered there would be at least one or two grams of limestone grit per bird still remaining in the gizzards at the time of the end of these tests. This would likewise affect the magnesium, giving a small balance, and the same holds good for the iron since the limestone used in these experiments contains 3 per cent iron. As a summary of the eight periods, there is an apparent shortage of phosphorus and a slight shortage of magnesium.

In this study of feeds from a mineral standpoint, it may be stated that mashes consisting of such mill feeds as middlings, also corn meal and ground oats must be used with feeds which bring up the percentage of phosphorus, magnesium, and calcium. Such feeds as meat and bone meal, or bone meal, and meat meal, should be added. Sour skim milk and buttermilk, if given in sufficient quantities, aids in making good the mineral shortage, as well as providing food hormone which has a stimulating effect upon the growth of the young as shown by work in this and other institutions.

THE MINERAL CONTENT OF OTHER FEED MIXTURES

It is desired here to take into consideration for study other feed mixtures for chick raising which we have found very satisfactory. These mixtures are as follows. Parts by weight.

MIXTURE 5

Peanut meal (fat ext.) 35 parts.
Ground corn, 65 parts.

At feeding, this mash is to be fed with milk and the chicks are to have milk to drink. The chicks are allowed to range on grassy runs. If milk is not available, 10 parts meat or blood meal should be added.

MIXTURE 6

Soybean meal (fat ext.), 35 parts.
Ground corn, 65 parts.

At feeding, this mash is to be fed with milk and the chicks are to have milk to drink. They are also to be allowed to run on grassy plots. If milk is not available, 10 parts meat or blood meal should be added.

MIXTURE 7

Ground corn, 28 parts.
Wheat middlings, 28 parts.
Ground oats, 28 parts.
Meat and bone meal, 16 parts.

At feeding, this mixture is to be used with sour milk and the chicks are to be given sour milk to drink. The chicks are to be allowed to run at large.

MIXTURE 8

Soybean meal (fat ext.), 30 parts.
Ground corn, 30 parts.
Wheat middlings, 25 parts.
Bone meal, 5 parts.
Meat and bone meal, 10 parts.

This mixture is to be used with sour skim milk, or with buttermilk, and the chicks given milk to drink.

MIXTURE 9

Ground oats, 1 part.
Ground corn, 1 part.
Peanut meal (fat ext.), 1 part.

This mixture is to be used with sour skim milk, or buttermilk, and the chicks given milk to drink. If milk is not available, 10 per cent meat meal should be added.

MIXTURE 10

Ground oats, 1 part.
Ground corn, 1 part.
Soybean meal (fat ext.), 1 part.

This mixture is to be used with sour skim milk or buttermilk and the chicks given milk to drink. If milk is not available, 10 per cent meat meal should be added.

The following table gives the comparative mineral study of the analyses of the bodies of the baby chick, the broiler, the hen and of the ten feed mixtures. Parts per hundred.

TABLE XII

MINERAL CONTENT. PARTS PER HUNDRED

Fowl or Mixture	K	Na	Ca	Mg	S	Cl	P	Fe
Baby chick	0.2922	0.2774	0.1978	0.0028	0.0107	0.1510	0.3550	0.0054
Broiler	.2380	.1580	1.0340	.0440	.3030	.0790	1.2880	.0056
Hen	.2750	.1640	1.2970	.0510	.3820	.2080	1.5100	.0066
Mixture 1	.6450	.4060	2.6154	.2796	.2570	.1440	1.8214	.0079
Mixture 2	.4090	.0410	.0193	.0827	.2000	.0670	.4280	.0023
Mixture 3	.4300	.4480	4.8126	.3049	.2380	.2470	2.8050	.1176
Mixture 4	.3940	.0270	.0270	.0992	.1950	.0600	.4090	.0025
Mixture 5	.6387	.1609	.1081	.2011	.2170	.0308	.4788	.0131
Mixture 6	.6429	.1922	.0892	.1914	.2573	.0366	.4540	.0131
Mixture 7	.5013	.1797	3.6421	.2455	.2181	.4747	1.4779	.0066
Mixture 8	.7391	.5502	2.7667	.3088	.2784	.4925	1.6568	.0183
Mixture 9	.6320	.1409	.0807	.1927	.2250	.0607	.4940	.0413
Mixture 10	.6360	.1701	.1140	.1535	.2630	.0570	.4703	.0151

A study of the mineral content of these various rations shows that none are deficient in potassium. All vegetation used for feed is apparently rich in this one element. Mixtures 2 and 4, all grain mixtures, are deficient in sodium and, to a slight degree, this is true of Mixture 9. All of the mixtures made up of grain or mill products are deficient in calcium, only those containing meat and bone meal, and bone meal have a sufficient supply of this mineral. The mixtures containing meat and bone meal, and bone meal namely, 1, 3, 7, and 8, have an excess of calcium. None of the mixtures are deficient in magnesium. All are apparently deficient in sulphur. Again, all of the purely grain mixtures are deficient in chlorine. All the grain and mill feed mixtures are deficient in phosphorus, only those containing meat and bone meal, and bone meal have sufficient phosphorus. Only the grain mixtures consisting of corn, wheat, and oats are deficient in iron, that is, Mixtures 2 and 4, all the balance having a surplus.

It must be kept in mind, however, that these comparisons are made comparing the mixture to the analyses of the chick when, in fact, it requires approximately three pounds feed to produce one pound of gain. Since mineral eliminated from the body, as a result of metabolism, is given off through the mucosa of the bowel, and through the kidneys, and since all this material is emptied into the cloaca, it is impossible to tell just how much is eliminated from the body, and how much remains in the intestinal tract unutilizable. However, it appears that feed without meat and bone meal, meat meal, bone meal, or milk, will be deficient in calcium and in phosphorus, with a possibility of a slight shortage in some feed combinations of chlorine. In mixtures of corn, wheat, and oats alone, there will be a deficiency in sodium. It is quite evident that mashes are essential in chick feeding as a supplement to the grain mixtures to furnish sufficient mineral salts which are essential for proper growth and development.

It must also be kept in mind that limestone grit, oyster shell, bugs and insects, as well as worms and other materials picked up by chicks on range aid in making good mineral shortage, but the exact amount is always problematical.

If the above feed mixtures were fed with milk, and the chicks given milk to drink, the mineral balance would be changed.

The average amount of milk to produce one gram of weight is found to be 7.49 grams, with mash and grain amounting to 2.91 grams. Taking this as the standard, the following mineral content in total feed taken in is had, that is, not taking into consideration green feed or materials secured in foraging. Each gram of mash requires 2.57 grams milk, and 0.34 gram green feed.

TABLE XIII

MINERAL CONTENT. PARTS PER HUNDRED

Fowl or Ration	K	Na	Ca	Mg	S	Cl	P	Fe
Baby chick	0.2922	0.2774	0.1978	0.0028	0.0107	0.1510	0.3550	0.0054
Broiler	.2380	.1580	1.0340	.0440	.3030	.0790	1.2880	.0056
Milk x2.57	.3880	.3700	.3932	.0047	.1090	.1670	.3495	.0092
Mixture 2	.4090	.0410	.0193	.0827	.2000	.0670	.4280	.0023
Mixture 4	.3940	.0270	.0270	.0992	.1950	.0600	.4090	.0025
Mixture 5	.6387	.1609	.1081	.2011	.2170	.0308	.4788	.0131
Mixture 6	.6429	.1922	.0892	.1914	.2573	.0366	.4540	.0131
Mixture 9	.6320	.1409	.0807	.1927	.2250	.0601	.4940	.0413
Mixture 10	.6360	.1701	.1140	.1535	.2630	.0570	.4703	.0151
S. oats x0.34	.0853	.0027	.0028	.0070	.1203	.0316	.0348	trace

The following table gives the total amount of mineral contained in each combination of feed mixture with milk and sprouted oats in the proportions it required, in these two tests, to produce one gram gain in weight.

TABLE XIV

MINERAL CONTENT. PARTS PER HUNDRED

Fowl or Ration	K	Na	Ca	Mg	S	Cl	P	Fe
Mixture 2	0.8823	0.4137	0.4153	0.0944	0.4293	0.2656	0.8123	0.0115
Mixture 4	.8673	.3997	.8355	.1109	.4243	.2586	.7933	.0117
Mixture 5	1.1120	.5336	.5041	.2128	.4463	.2294	.8631	.0223
Mixture 6	1.1162	.5649	.4852	.2031	.4866	.2352	.8383	.0223
Mixture 9	1.1053	.5136	.4767	.2044	.4543	.2587	.8783	.0505
Mixture 10	1.1093	.5428	.5100	.1652	.4923	.2556	.8546	.0243
Baby chick	.2922	.2774	.1978	.0028	.0107	.1510	.3550	.0054
Broiler	.2380	.1580	1.0340	.0440	.3030	.0790	1.2880	.0056

In an analysis of Table XIV it is noted that only calcium and phosphorus are not supplied in sufficient quantities. As stated previously these mixtures are fed with milk and sprouted oats in the proportion required as an average in these two feeding tests here given. It yet appears that it is necessary to add either bone meal, or bone and meat meal, to the mixture to bring these requirements up to the standard. Grit and oyster shell will supply the calcium, but not the phosphorus deficiency.

During the year 1917 feed Mixture 7 was tried out at one of the Branch Experimental Farms. These birds were of the Single Comb Rhode Island Red. The hens were set and the chicks were brooded in the combination sitting and brooding coop*, the hen being confined to the coop and the chicks allowed to run at large. One-half the broods received milk in the mash and the other half no milk at all. Both lots secured worms, bugs and insects, as well as green feed, in ranging a short distance from their coop.

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*Described in circular No. 6 from this Station.

TABLE XV

RESULTS OF NINE FLOCKS RECEIVING MIXTURE 7 WITH MILK

Date†	Number at Start	Number at End	Average Weight at 8 Weeks
3-20	14	13	1.44‡
3-26	13	11	1.55
4- 2	13	13	1.50
4-11	12	11	1.15
4-19	11	8	1.43
4-19	11	8	1.36
4-19	14	12	1.39
5- 3	10	4	.87
5-21	18	15	1.32
	—	—	——
Totals	116	95	12.01
Average	14	10.5	1.33

†Date of commencement.
‡Weight in decimal pounds.

There are nine test lots given here with the mash mixture, with milk and milk to drink. The average mortality was 25 per cent. The average weight of the chicks at the end of the eight weeks was 1.33 pounds.

The following control lots were treated and fed exactly the same as those recorded in Table XV, except that they did not receive any milk either in the feed or as drink.

TABLE XVI

CONTROLS TO LOT RECORDED IN TABLE XV

Date	Number at Start	Number at End	Average Weight at 8 Weeks
3- 3	8	4	1.15
3-20	13	13	1.32
3-26	10	9	1.50
4- 2	13	9	1.14
4-11	10	6	.95
4-21	6	6	1.13
5- 3	10	7	1.05
5-24	18	13	.98
	—	—	——
Totals	88	67	9.22
Averages	11	8.7	1.15

There are eight lots of chicks given here that were fed the same as those given in Table XV, with the exception that they did not get any milk. These, as well as the former lot, received meat meal in the feed mixture. The average of the water lot at eight weeks was 1.15 pounds each. The milk lot averaged 13 per cent greater weights than the water lot.

During the year 1916 feed Mixture 10 was tried out on one of the Branch Experimental Farms. This work was done on a flock of Buff Plymouth Rock. The hens were set, and the chicks were brooded in a combination sitting and brooding coop. The hen was confined to her run and the chicks were allowed to run at large. Part of the flocks were given milk in mash, and milk to drink, otherwise the same care and treatment.

TABLE XVII

FLOCK GIVEN FEED MIXTURE 10

Date	Number at Start	Number at End	Average Weight at 8 Weeks
7-14	17	16	1.27
5-10	28	26	1.40
4-26	22	20	1.31
5-10	24	17	1.51
4-15	22	20	1.56
4-15	7	3	1.26
3-19	18	16	1.42
7-12	19	17	1.01
8-17	17	10	1.04
Totals	175	145	11.78
Averages	19	16	1.30

Table XVII is the result of nine milk-raised flocks. There was an average of 19 chicks at the start, and 16 at the finish. The average weight of all chicks at the end of the eight weeks' period was 1.30 pounds. The per cent raised was 82.8.

The following table gives the results of four water fed lots. These flocks were likewise Buff Plymouth Rocks, and received the same care and feed as those reported in Table XVIII, except that they did not receive any milk, either in the feed or to drink. These chicks received feed Mixture 10.

TABLE XVIII

CONTROLS TO FLOCK RECORDED IN TABLE XVII

Date	Number at Start	Number at End	Average Weight at 8 Weeks
2- 5	8	8	.75
3-20	13	13	1.32
4- 4	22	8	.76
3-24	32	30	.70
Totals	75	59	3.53
Averages	18	14.7	.88

There are four flocks recorded here, an average of 14.7 birds at the finish, an average percentage raised 78 or 6.2 per cent less raised, as compared to the milk fed flocks run at the same time. The average weight of all birds in the tests was 0.88 pound, or 32 per cent in favor of milk feeding with this feed mixture. The variation is greater than in those chicks fed with Mixture 7, which mixture contains 16 per cent meat meal.

During the year 1916 there were twenty-two test flocks run at one of the Branch Experimental Farms with White Plymouth Rocks. These birds received Feed Mixture 9 with mash, mixed with milk, and milk for the birds to drink.

TABLE XIX

RESULTS OF FLOCKS RECEIVING MIXTURE 9

Date	Number at Start	Number at End	Average Weight End 8 Weeks
6–29	11	10	1.10
6–28	9	9	.91
7– 5	6	4	.95
7– 3	10	9	.81
7–13	7	7	1.05
7– 9	13	11	.89
6–26	11	11	.90
6–25	6	6	1.08
6–25	14	12	.86
5–11	7	6	1.16
5–12	5	5	1.02
4–25	11	6	1.09
4–25	9	4	1.00
4–25	11	11	.99
4–13	7	6	1.36
4– 8	19	18	1.14
4– 8	19	8	1.30
Totals	175	143	17.61
Averages	10	8	1.03

In this test the average weight at the end of eight weeks was 1.03 pounds. The mortality was 10 per cent. This is milk fed work.

During the year 1917 there were five test lots of chicks with ration 9 with chicks from the same White Plymouth Rock flock as those with the same mixture, plus milk run on the previous year. The hens were set and the chicks brooded similar to those of the year before. The following is a tabulation of these results:

TABLE XX

CONTROL TO FLOCKS RECORDED IN TABLE XIX

Date	Number at Start	Number at End	Average Weight at End of 8 Weeks
2–15	10	7	.82
4–18	13	11	.56
4–23	10	7	.60
4–18	11	10	.58
5–22	13	10	.70
	—	—	——
Totals	57	45	3.26
Averages	11.4	9	.65

In this series of tests, the average weight at the end of eight weeks was but 0.65 pounds. There was 78 per cent raised. The birds the previous year averaged 1.03 pounds each or 36 per cent greater gain in favor of the sour skim milk feeding.

The results this far favor the greater development of those birds receiving nearer the required amounts of mineral elements. These are the birds receiving in their mash mixture animal food as meat meal, meat and bone meal, or are fed milk; and in addition green feed, limestone grit and crushed oyster shell.

SUMMARY

It is noted in the alanyses of the bodies of birds at different ages in their life beginning when first hatched that as the bird grows larger, that the quantity of calcium, magnesium, and phosphorus is increased. As the bird grows there is a greater proportionate amount of bony structure. There is also an increase of sulphur due to the fact that as the baby chick grows its downy coat is changed to a coat of feathers. There is also an increase in chlorine.

In these studies seventy-three complete mineral analyses of feeds were made besides analyses of excreta.

In the first tests, the experimental birds were given grain mixtures consisting of oats, corn, and wheat. The mash contained wheat middlings, bone meal, meat and bone meal, and corn meal. In addition they were given all the sour skim milk they would drink, finely cut green feed in the form of rape, also oyster shell, and limestone grit. In a study of the balances from the table of intake and outgo there appears ample potassium, sodium, calcium, sulphur, chlorine, and iron but a slight deficiency of magnesium and phosphorus.

Feed Mixture 1 has a nutritive ratio of 1:3.2; 2, 1:7.4; 3, 1:2.2, and

4, 1:7.6, which from a protein-carbohydrate standpoint should be ample. It contained approximately a mineral balance. There was required to produce one pound of gain 2.91 pounds grain and mash, 7.49 pounds sour skim milk (reduced to dry matter would be 0.74 pound) and 1. pound green feed (reduced to dry matter would be 0.1 pound), or a total of 3.75 pound feed.

The feeds here studied which are richest in calcium are oyster shell, limestone grit, soybean meal, velvet bean meal, meat and bone meal, bone meal, and eggs in shells. Those richest in magnesium are oyster shells, limestone grit, peanut meal, soybean meal, velvet bean meal, meat and bone meal, bone meal, and wheat middlings. Those richest in phosphorus are eggs in shells, peanut meal, soybean meal, velvet bean meal, meat and bone meal, oats, bone meal, wheat middlings, corn, and wheat. Iron does not appear to be deficient in most feeds. Chlorine is most abundant in eggs in shells, velvet bean meal, and in meat and bone meal. Sodium is most abundant in eggs in shell, peanut meal, soybean meal, meat and bone meal, bone meal, and wheat middlings. Most cereals and their by-products are deficient in chlorine, sodium, and calcium. Potassium does not appear to be deficient in any of the feeds.

Feed mixtures such as grains and their by-products need such feed stuffs as meat scrap, bone meal, meat and bone meal, milk, and oyster shell and limestone grit, with 1 per cent sodium chloride to make good the deficiency in phosphorus, mangesium, calcium, chlorine, and sodium. Of the vegetable products both soybean meal and peanut meal are particularly rich in sodium, potassium, and magnesium. The excellent results which have been obtained by these two feeds are, in part, due to this fact.

In Feed Mixtures 5 and 6 we have introduced the most simple mixtures which have given very gratifying results in those tests where these feeds were given with milk. There is here a fair amount of the most vital minerals, besides essential amino acids, the only minerals deficient is chlorine which can be compensated for largely by adding ¾ per cent sodium chloride. These mixtures consist of but two feeds, viz.: corn meal with, in the one case, soybean meal and in the other peanut meal. In Mixture 7 the mineral and protein deficiencies are compensated for by the meat and bone meal. It would be expected that better results would be attained by Mixture 8 which has, in addition to the fat extracted, soybean meal, bone meal and meat and bone meal. Ground oats is added in Mixtures 9 and 10 which no doubt adds to the palatability and lends a greater variety.

Mashes or ground feed mixtures are essential in chick raising to furnish sufficient mineral salts which are essential for proper growth. In tests with Feed Mixtures 5 to 10 the chicks were allowed the run of the grassy yards and were not given grain mixtures. The mortality was not greater than other tests where grain mixtures were fed. It is not essential to give grain mixtures where chicks run at large and can secure bugs, waste grain and other feeds.

In nine flocks of Single Comb Rhode Island Red chicks given Mixture 7 with milk and with milk to drink the average weight per chick at the age of eight weeks was 1.33 pounds, while eight flocks given the same feed but did not receive milk averaged 1.15 pounds each. The milk lot averages 13 per cent greater in weight than the water lot. This difference in weight is due partly to the increased minerals required. The fat remaining in the skim milk furnishes needed fat soluble vitamine, and the milk also contains suitable proteins.

Nine flocks of Buff Plymouth Rock chicks were given Mixture 10 with milk and with milk to drink. These birds averaged, at the end of eight weeks, 1.30 pounds while four flocks given the same mixture but no milk to drink averaged but 0.88 pound each. The milk lot averaged 47 per cent greater in weight than the water lot.

Twenty-two flocks of White Plymouth Rock chicks were given Mixture 9 with milk and milk to drink, at the end of eight weeks these chicks averaged 1.03 pounds each while five flocks given the same feed but no milk averaged 0.65 pounds each. The milk lot averaged 58 per cent greater in weight than the water lot.

The results thus far show greater development in those birds receiving nearer the required amounts of mineral matter. These are the ones receiving animal feeds as bone meal, meat and bone meal, meat scrap, or milk together with green feed and crushed oyster shell and limestone grit.

THE POTENTIAL ALKALINITY AND POTENTIAL ACIDITY OF SOUTHERN POULTRY FEEDS*

IMPORTANCE OF THESE STUDIES

In the future balancing of rations, there is a possibility that some of the expensive ingredients will be "cut out" as we find just those elements which are essential to produce the greatest results, and just those feeds that contain them. In other words, in the future we are going to more intelligently and more economically feed our birds for the results we are trying to attain.

It is essential to take into consideration many things in compounding the ration or feed mixture, and that simply so much protein to a certain amount of carbohydrates and fat as expressed by the nutritive ratio is insufficient. Aside from those points primarily discussed in this bulletin, the following should be taken into consideration:

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*The analytical work was done by Dan. M. McCarty and Jno. E. Ivey of this station

1. Proteins high in certain amino acid content.
2. Sufficient amounts of vitamine, especially fat soluble.
3. Sufficient amounts of essential minerals.
4. A ration that is not excessively acid.

Of the seventeen amino-acids found in protein, six or seven are found to be indispensable for greatest results. Protein feeds high in these compounds can be selected, and those low can be rejected. It was found that lysine and cystine are essential for proper growth of the bird and the feeds containing the greater amounts of these compounds are leguminous seeds such as soybeans and peanuts, also animal foods such as eggs, milk, meat and bone meal. They are found to a less extent in grains such as oats, wheat and rye but are absent in corn. Other amino-acids so essential are tryptophane and histidin which are essential for maintenance of life, and the feeds in which these compounds are abundant are seeds of leguminous plants such as peas, soy beans, and peanuts. To a less extent they are found in grain though absent in corn. They are also found in the animal feeds, such as egg, milk, and meat scrap.

For vitamines of the fat soluble kind we look principally to eggs, milk, and to the leafy vegetables, the legumes being richer in this element than some of the other vegetables, such as rape and cabbage. The thin leafy vegetables contain much more than thick storage leaves like cabbage and are hence a more valuable green feed. It requires from 25 to 40 pounds of vegetables to contain as much vitamine as one pound of egg or one pound of whole milk. Skim milk has been robbed of much of its fat soluble vitamine, for this substance is found in the fat.

Sufficient amounts of essential minerals can be furnished by meat and bone meal, bone meal, oyster shell, limestone grit, and sodium chloride in the mash. The minerals most feeds are short in are sodium, calcium and chlorine.

There is a possibility of selecting feeds that are excessively acid in the body, and overtaxing the system to neutralize them, profound results may follow.

Interest in acid-base balance of diets has increased greatly in recent years by those who have been scientifically studying these problems. It has been found that meats and cereals like wheat, corn, and oats have a preponderance of acid-forming elements, while on the other hand, fruits and vegetables have an excess of base forming elements. The influence of excessive acid feeds, or excessive alkaline feeds upon the body, has been proven by the feeding of highly acid feeds which increase acid urines and excessive feeding of alkaline feeds, which produce an excess of alkaline urines.

There is evidence that excess acid forming diets eaten for some period of time is productive of undesirable results and there is a possibility that the continued feeding of acid-forming diets may lead to a greater susceptibility to disease.

A common defect is the feeding of proteins and fats far in excess of the body's needs, and as fats and proteins are expensive materials, work is in progress in this laboratory to determine just the smallest amounts of proper kinds of proteins and the feeds that contain just these desired proteins with their proper amino-acids.

OUR PRESENT PROBLEM

In the work that is presented here our problem was to determine the potential acidity and potential alkalinity of these feeds based on previous mineral analyses. So far as we know this has never been done for poultry feeds. Our second problem was to determine the acid-base balance of the feed mixtures used in our experimental feeding work in broiler prduction, giving some of the feeding results and suggesting ways of overcoming excess acidity of feeds.*

EXPERIMENTAL METHODS

The baby chicks were produced from a single flock of pure-bred Single Comb White Leghorns bred at the Station and College poultry plant, and hatched in the same incubator. Each lot was housed under similar oil burner hovers of 100-chick capacity. The experiment was carried on in periods of eight weeks each, and extended over 3 periods, or 24 weeks.

The samples of feeds for analysis were obtained, as composite samples, from the various bags of feed used in the experiment.

The potential acidity and potential alkalinity was estimated from the mineral analysis of the above feed samples.

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*This same work is now progressing with laying hens.

TABLE XXI

POTENTIAL ACIDITY AND POTENTIAL ALKALINITY OF SOUTHERN POULTRY FEEDS

(Results expressed in cc. excess per pound feed)

Feed	Base	Acid
Corn meal, bolted		21.75
Pinhead oats		121.94
Whole wheat		79.09
Wheat midlings	206.58	
Bone meal	2104.60	
Meat and bone meal	565.13	
Rolled oats		108.21
Whole corn		44.76
Hulled oats		79.17
Velvet bean meal	62.17	
Soybean meal	156.01	
Peanut meal	94.90	
Skim-milk	23.18	
Green rape	15.33	
Lime stone grit	11943.80	
Oyster shell	8782.10	
Dried buttermilk	803.86	
Digester tankage	789.89	
Dried blood	63.68	
Egg, including shell		2.55
Peas, dried	24.00	
Potatoes, sweet	24.00	
Potatoes, white	26.00	
Rice		43.00
Spinach	122.00	
Turnips	25.00	
Beans, dried	79.00	
Beets	50.00	
Bread, hard		38.00
Cabbage	22.00	
Carrots	38.00	
Fish, dried		36.00
Hominy		24.00
Lettuce	27.00	

FEED MIXTURES

The following mixtures were used in these experiments, the proportions being given in weight:

TABLE XXII

ACID-BASE BALANCE OF POULTRY RATIONS

(Results expressed in cc. excess per pound feed)

Ration Number	Kinds of Feed	Amount	Acid	Base	Per Cent Protein Calories
1	SCRATCH FEED				
	Corn	100	--------	--------	--------
	Oats	100	61.96	--------	12
2	MASH FEED				
	Wheat middlings	35	--------	--------	--------
	Corn meal	30	--------	--------	--------
	Ground oats	35	--------	--------	--------
	Dried buttermilk	35	--------	231.4	27
3	Wheat middlings	35	--------	--------	--------
	Corn meal	30	--------	--------	--------
	Ground oats	35	--------	--------	--------
	Meat and bone meal	20	--------	125.9	30
4	Wheat middlings	35	--------	--------	--------
	Corn meal	30	--------	--------	--------
	Ground oats	35	--------	--------	--------
	Digester tankage	18	--------	153.7	30
5	Wheat middlings	35	--------	--------	--------
	Corn meal	30	--------	--------	--------
	Ground oats	35	--------	--------	--------
	Soybean meal	24	--------	63.9	30
6	Wheat middlings	35	--------	--------	--------
	Corn meal	30	--------	--------	--------
	Ground oats	35	--------	--------	--------
	Peanut meal	41	--------	54.5	19
7	Wheat middlings	35	--------	--------	--------
	Corn meal	30	--------	--------	--------
	Ground oats	35	--------	--------	--------
	Blood meal	14	--------	41.21	31

DISCUSSION

The low caloric value of peanut meal ration (Ration No. 6) is due to the fact that the meal was made from peanuts and hulls not fat extracted under which conditions we find 40.40 per cent fat.

The caloric value of all the animal food rations—Nos. 2, 3, 4, and 7—are about the same; containing 27 to 31 per cent protein calories.

The soybean meal ration is in the same column so far as protein calories are concerned.

The grain feeds have less protein caloric value, having only 12 per cent.

Dried buttermilk functions as base. The meat scrap and bone meal, and digester tankage are base on account of the large amount of calcium of the bone. Blood is normally alkaline due to the sodium salts.

The grain ration is 61.96 cc. acid per pound. The mashes are all alkaline or base. Those containing milk or bone, as Rations 2, 3, and 4, run high in base elements, while the blood meal runs only 41.21 cc. base which compares favorably with the two rations containing no animal food but protein from leguminous sources.

If we estimate the per cent protein from peanut meal fat extracted, i.e., by using 7 per cent fat, as would have been the case if fat extracted peanut meal had been used, then the per cent protein calories will be 28 instead of 19. This compares favorably with fat extracted soybean meal ration.

TABLE XXIII

SHOWING RELATION OF PROTEIN CALORIES, AMOUNT FEED, AND ACID BASE BALANCE, TO POUND GAIN

(With Single Comb White Leghorns)

Ration Number	Period*	Amount Feed Consumed			Amount Feed to Make Pound Gain	Per Cent Gain	Excess Acid	Excess Base	Per Cent Protein Calories	Test Protein
		Mash	Grain	Total						
1 and 2	First	23.3	15.4	38.7	3.0	800†	-------	114.6	21	Buttermilk
	Second	35.2	28.6	63.8	4.2	200	-------	99.8	20	
	Third	30.3	54.5	84.8	6.0‡	63	-------	42.8	17	
1 and 3	First	22.8	18.7	41.5	2.7	905	-------	41.2	21	Meat and bone meal
	Second	36.7	35.3	72.0	4.9	137	-------	33.8	21	
	Third	34.8	61.9	96.7	4.9	50	-------	5.6	17	
1 and 4	First	23.6	20.0	43.6	1.8	1142	-------	54.8	21	Digester Tankage
	Second	17.5	23.7	41.2	6.4	141	-------	29.6	19	
	Third	14.3	22.2	36.5	6.0	62	-------	22.5	19	
1 and 7	First	19.7	20.8	40.5	2.1	1300	11.7	-------	21	Blood meal
	Second	33.8	33.1	66.9	5.8	83	9.8	-------	21	
	Third	26.1	48.7	74.8	7.0	49	25.8	-------	18	
1 and 5	First	19.5	14.5	34.0	2.9	900	-------	10.2	22	Soybean meal
	Second	31.3	36.9	68.2	5.9	133	4.1	-------	20	
	Third	30.0	56.9	86.9	8.4	51	18.4	-------	18	
1 and 6	First	32.7	21.9	54.6	3.1	800	-------	7.8	16	Peanut meal
	Second	30.9	27.3	58.2	4.7	206	0.1	-------	15	
	Third	10.4	48.4	58.8	5.3	59	41.3	-------	13	

*Each period consists of eight weeks

†Computed on the basis of the weight of the baby chick at hatching and at the beginning of each period

‡In the latter part of the third period, in all the flocks receiving animal food, egg production commenced. As egg production commenced it is noticed that the birds consumed relatively more mash which has a tendency to lessen the acid balance. If not in egg production, less mash and more grain is consumed, which increases the acid balance.

DISCUSSION

Many interesting things are brought out by this table. The greatest gain in weight in chicks is during the first eight weeks, or first period. Following the first period, their increase in weight gradually decreases during the balance of the two periods.

The amount of feed to produce a pound of gain gradually grows greater as the bird becomes older.

In these tests the bird gradually consumes more grain as it grows older. Likewise it was found that as a pullet comes into laying, it consumes a greater proportion of mash and again slackens in its mash consumption as it goes into a non-laying period.

The consumption of more grain and less mash has a tendency to lessen the base balance, or, if acid, has a tendency to increase the acid balance.

It was noted that the cereals are decided acid while the by-products from the legumes are of a base reaction. Those feeds containing by-products such as soybean meal and peanut meal have a tendency to add to the base balance.

It is noted that those rations containing buttermilk, digester tankage, and meat scrap and bone meal, give a base balance in all cases.

The soda in the blood makes the blood meal base, but not so strongly so as digester tankage or meat and bone meal containing much bone. Bone is rich in calcium and also contains other bases as sodium. As stated before milk functions as base.

In this work no account has been taken of the amount of calcium taken into the crop as grit and oyster shell. This will in all probability overcome the acid reaction, though without definite data this is a mere guess.

In a large table of studies of rations furnished by the medical staff of the U. S. Army the per cent protein calories ran from 10 to 18. In this work, estimating the actual intake in each period, the per cent protein calories ran from 13 to 22.

There is a possibility, however, that we will need to pay more attention to the source, or kinds and quantities, of protein calories since we have shown in this work that those birds which received animal food, and this includes milk, tankage, meat and bone meal, and dried blood were prepared by the storing up of the proper potential energy, to put these pullets into heavy egg production very young, while those birds that did not receive animal food of any kind were not prepared and did not go into early heavy egg production.

FEED MIXTURES USED IN SECOND SECTION OF WORK

The following summary of work done in this laboratory with Single Comb White Leghorns and reported under heading of Mineral Intake and Outgo in Journal of Agricultural Research*.

TABLE XXIV

FEED MIXTURES

Ration Number	Kinds of Feed	Amount	Acid	Base	Per Cent Protein Calories
8	Rolled oats	8			
	Wheat middlings	8			
	Meat and bone meal	2			
	Bone meal	1		128.3	29
9	Cracked wheat	3			
	Cracked corn	2			
	Pinhead oats	1	67.2		13
10	Wheat middlings	6			
	Corn meal	3			
	Meat and bone meal	3			
	Bone meal	1		382.6	30
11	Whole wheat	3			
	Cracked corn	2	67.2		13
	Hulled oats	1			

*B. F. Kaupp, Journal Agricultural Research, Vol. XIV, No. 3, July 15, 1918

TABLE XXV

SHOWING RELATION OF PROTEIN CALORIES, AMOUNT FEED, AND ACID BASE BALANCE, TO POUND GAIN

(With Single Comb White Leghorns)

Amount feed consumed first period. (From baby chicks to 8 weeks old)

Kind	Amount	Acid Excess	Base Excess	Per Cent Protein Calories
Milk	52.30		1212.31	
Ration No. 8	2.42		310.48	
Ration No. 9	8.42	565.82		
Ration No. 10	4.30		1645.18	
Ration No. 11	5.98	401.85		
Rape	7.81	119.72		22

Amount feed per pound gain, 3.4; per cent protein calories 22.
Per cent gain, 686. Excess base intake, 2320.02 c. c.
Excess base intake per pound feed, 85.5 c. c.

GENERAL SUMMARY

From Table XXI we note that the grains, such as corn, wheat, and oats, as well as egg (including the shell), rice, bread, fish, and hominy, are acid. The wheat middlings, in six analyses, on account of the high sodium and potassium content, is base. Green feeds such as rape, cabbage, carrots, beets, turnips, potatoes, spinach, and lettuce are base. Seeds of the legumes such as velvet bean, soybean, peanut, and peas are base. Bone meal on account of its high calcium content as well as other base forming elements, is highly base. Limestone grit is very highly base so also is crushed oyster shell, though to a less extent. The animal feeds containing bone, such as meat and bone meal, and digester tankage, are base. The calcium of the egg shell does not quite overcome the acid of the albumin of the egg. Dried milk functions as base for the lactic acid is oxidized to carbonic acid which is thrown off by the lungs, leaving the basic residue of mineral salts. Dried skim milk or dried buttermilk is therefore quite base in function. Dried blood, on account of its magnesium, calcium, and sodium content, is moderately base.

In these studies there have been arranged eleven feed mixtures for acid-base studies. The first seven are North Carolina Experiment Station formulæ and the last four are those of Professor Rice. The mixtures that contain considerable amounts of either dried milk, meat and bone meal, or digester tankage are quite base. The mixture containing soybean meal is approximately as much base as the grain mixture is acid so that equal amounts would approximately balance so far as acid-base content is concerned. The peanut meal mixture is slightly below the soybean meal mixture and the blood meal comes slightly below the peanut meal.

We note from Table XXII that the grain mixture contains 12 per cent protein calories and the ground feed mixtures contain from 19 per cent in the case of the non-fat extracted peanut meal mixture to 31 per cent in the case of the blood meal ration. We note by a study of Table XXIII which gives the total intake of each mixture for each period that the final percentage protein calories run from 13 to 22. As a comparison of rations for humans, we may again refer to the study of army rations during the late World War in which the percentage protein calories ran from 10 to 18. Dr. Osborne (Osborne and Mendel, J. Biol. Chem. 1916, XL, 16) found that 12.5 per cent protein calories produced maximum growth in rats. The indications are that the kinds as well as the quantities of proteins are essential factors. While the kinds of amino-acids and vitamines are important factors in addition to kinds and quantities of minerals, there is a possibility that there are other factors undiscovered, which have a profound bearing on growth, egg production, and the preparing of pullets, by aiding the storing up of potential energy, for early and heavy egg production. Other data shows that pullets grown on range or in confinement, and not receiving animal food of any kind though the protein calories were above those indicated in comparison rations given above, were not prepared for early heavy egg production and do not show high egg yields until animal food of some kind has been added. In this instance this was the soybean meal and peanut meal lots. In the second and third periods the balance of intake was acid.

Further studies are being made to determine if acid feeds will in any way interfere with either growth, or egg production. In these studies Rations 5 and 6 can be made base by the addition of ground limestone or ground oyster shell. The amounts to be added would depend upon the proportions in which the mash and grain was fed.

In the second section of this work we find, by a study of Table XXIV, that the grain Rations Nos. 9 and 11 are acid and the mash is base. In these mash mixtures there has been added both bone meal and meat and bone meal. Wheat middlings also aid in overcoming the acidity of corn meal and of rolled oats.

In this second, work was run with Single Comb White Leghorns, the first period extending from the time of hatching to eight weeks of age. In this case the total intake excess was base. The per cent protein calories was 22.

CONCLUSIONS

Grain mixtures as ordinarily used in poultry feeding are acid.

Mash mixtures containing sufficient quantities of digester tankage, meat and bone meal, dried milk, or dried blood, will be base.

Acid balances of feed mixtures can be overcome by the addition to mashes of dried milk, digester tankage, meat and bone meal, bone meal, dried blood, or ground limestone or oyster shell. Green feed, milk to drink, and limestone and oyster shell grit also aid in overcoming the acid balance of grain mixtures.

B

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STUDIES ON THE PHYSIOLOGY OF SOME PLANT PATHOGENIC BACTERIA

NORTH CAROLINA
AGRICULTURAL EXPERIMENT STATION

CONDUCTED JOINTLY BY THE STATE DEPARTMENT
OF AGRICULTURE AND THE NORTH CAROLINA STATE
COLLEGE OF AGRICULTURE AND ENGINEERING

RALEIGH

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Frederick A. Wolf

I. Solid Culture Media with a Wide Range of Hydrogen or Hydroxyl Ion Concentration*

A considerable number of investigations, made during the past few years, have extended our knowledge of the profound influence exerted by acids and alkalis upon the growth of microorganisms. This is especially true in the case of such fungi and bacteria as lend themselves readily to cultivation on artificial media. Investigations have also clarified many problems related to these media themselves, such as the influence of acids and alkalis on colloidal hydration and jellification, the buffer action of proteins and salts, the devising of improved colorimetric and electrometric technic for the measurement of hydrogen and hydroxyl ion concentration, etc. The point covered in the present study, which grew out of an attempt to determine the limit of tolerance of certain organisms to acid and alkali on solid media, does not appear to have been brought out in any foregoing investigation. In previous studies use has been made of liquid media for very tolerant organisms, even for forms which are known to thrive best on solid media, since it has been impossible to make agar or gelatin with high pH values solid. It is the present purpose, therefore to show that acids and alkalis need not materially modify the physical properties of agar and gelatin media within, and even far beyond, the limits of tolerance of any living organism.

METHODS

The media were prepared by adding either 1 or 2 per cent commercial agar or 10 or 15 per cent bacto-gelatin to a bouillon consisting of 0.3 per cent Liebig's beef extract, 1 per cent Armour's peptone and 0.5 per cent sodium chlorid. They were then heated in an autoclave, flasked, and sterilized, for fifteen minutes at 15 pounds pressure in the case of agar, and 10 pounds pressure in the case of gelatin. No attempt was made to adjust the reaction of the media to neutrality prior to sterilization. The acid used was hydrochloric, with a specific gravity of 1.20 or it possessed an HCl concentration of 39.11 per cent. The sodium hydroxid had a specific gravity of 1.226 or an NaOH concentration of approximately 20 per cent. Strong acid and alkali were employed to eliminate the factor of dilution of the media. Upon removal from the autoclave the agar was cooled to about 50°C. and the gelatin to about 40°C. before the addition of appropriate quantities of acid or alkali, and were maintained at these temperatures while 10 cc. portions were withdrawn with a pipette and put into test tubes. The acid or alkali was added to these 10 cc. portions with a 1 cc. pipette graduated in tenths. After the addition of the acid or alkali the tubes were well agitated and were further cooled with the results indicated in the tabulations which follow.

EXPERIMENTAL

Only those proportions of agar or gelatin which are commonly employed in making culture media were used in this study, but they indicate, as would be anticipated, that the jellifying power is modified by the proportion of colloidal material added. The results with 1 and 2 per cent agar are shown in Table 1.

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*Reprinted from Jour. Bact. 6: No. 3, 325-330. 1921.

It will be noted that the limits of solidification of 1 per cent agar are approximately 5.11 per cent acid and 0.39 per cent alkali, whereas 2 per cent agar does not lose its jellifying power until 6.51 per cent acid or 0.58 per cent alkali has been added. A better appreciation of the degree of acidity and alkalinity of these limits can be gained when they are compared with pH values determined by the colorimetric method of Clark and Lubs (1917). It was found that the addition of 0.1 cc. of HCl to 10 cc. of agar gave a concentration of about pH 1.4 and 0.03 cc. NaOH a concentration of about pH 9.2. In reaching the limits in 2 per cent agar it will be seen that 20 times this volume of acid and 9 times this volume of alkali were employed. Manifestly, these limits are only approximate and could be more accurately determined by improved technic. They are, however, far beyond the limits of tolerance of microorganisms and are intended only to show that hydrogen or hydroxyl ion concentration need not be limiting factors in the preparation of solid agar media.

Table 1.—Effect of Acid and Alkali on Solidification of Agar

1 Per Cent Agar						2 Per Cent Agar					
Agar	HCl		NaOH		Physical State, 20° C.	Agar	HCl		NaOH		Physical State, 20° C.
cc.	*cc.*	*Per Cent*	*cc.*	*Per Cent*		*cc.*	*cc.*	*Per Cent*	*cc.*	*Per Cent*	
						10	2.0	6.51	------	--------	Semisolid
						10	1.7	5.68	------	--------	Solid
10	1.5	5.11	------	--------	Semisolid	10	1.5	5.11	------	--------	Solid
10	1.2	4.19	------	--------	Solid	10	1.2	4.19	------	--------	Solid
10	1.0	3.55	------	--------	Solid	10	1.0	3.55	------	--------	Solid
10	0.7	2.55	------	--------	Solid	10	0.7	2.55	------	--------	Solid
10	0.5	1.86	------	--------	Solid	10	0.5	1.86	------	--------	Solid
10	0.3	1.14	------	--------	Solid	10	0.3	1.14	------	--------	Solid
10	0.1	0.38	------	--------	Solid	10	0.1	0.38	------	--------	Solid
10	--------	--------	0.1	0.19	Solid	10	--------	--------	0.1	0.19	Solid
10	--------	--------	0.2	0.39	Semisolid	10	--------	--------	0.2	0.39	Solid
10	--------	--------	0.3	0.58	Liquid	10	--------	--------	0.3	0.58	Semisolid

All of the agar media in these series appear to be able to remain solid for an indefinite period when they are maintained at room temperature. If, however, they are autoclaved and then cooled all of the acid media were found to remain liquid, whereas the tubes of 1 per cent agar with 0.1 cc. NaOH and 2 per cent agar with 0.1 and 0.2 cc. NaOH became solid again.

Considerably larger amounts of acid or alkali must be added to 10 and 15 per cent gelatin to destroy the jellifying power as shown in Table 2.

In the case of 10 per cent gelatin the limits are seen to be about 9.02 per cent HCl and between 3.33 and 4 per cent NaOH, and of 15 per cent gelatin between 9.02 and 10.15 per cent HCl and about 4 per cent NaOH. When these media were examined after having been maintained in an ice chest at about 10°C. for twelve hours, all of those to which more than 1 cc. of NaOH had been added were found to have become liquid, and a heavy whitish precipitate had formed. All of the tubes to which HCl had been added were still solid, however. All of the tubes containing media which had remained solid were placed in boiling water until the media had liquified, whereupon they were again cooled to 7.5°C. The alkaline gelatin again solidified, but 1.5 cc. of HCl in 10 per cent gelatin and 1.7 cc. in 15 per cent gelatin were now the limits of the jellifying power.

Table 2.—Effect of Acid and Alkali on Solidification of Gelatin

10 Per Cent Gelatin						15 Per Cent Gelatin					
Gelatin	HCl		NaOH		Physical State, 7.5° C.	Gelatin	HCl		NaOH		Physical State, 7.5° C.
cc.	*cc.*	*Per Cent*	*cc.*	*Per Cent*		*cc.*	*cc.*	*Per Cent.*	*cc.*	*Per Cent*	
						10	3.5	10.15	------	--------	Liquid
10	3.0	9.02	------	--------	Semisolid	10	3.0	9.02	------	--------	Semisolid
10	2.5	7.82	------	--------	Solid	10	2.5	7.82	------	--------	Solid
10	2.0	6.51	------	--------	Solid	10	2.0	6.51	------	--------	Solid
10	1.7	5.68	------	--------	Solid	10	1.7	5.68	------	--------	Solid
10	1.5	5.11	------	--------	Solid	10	1.5	5.11	------	--------	Solid
10	1.2	4.19	------	--------	Solid	10	1.2	4.19	------	--------	Solid
10	1.0	3.55	------	--------	Solid	10	1.0	3.55	------	--------	Solid
10	0.5	1.86	------	--------	Solid	10	0.5	1.86	------	--------	Solid
10	--------	--------	0.5	0.95	Solid	10	--------	--------	0.5	0.95	Solid
10	--------	--------	1.0	1.82	Solid	10	--------	--------	1.0	1.82	Solid
10	--------	--------	1.2	2.14	Solid	10	--------	--------	1.2	2.14	Solid
10	--------	--------	1.5	2.61	Solid	10	--------	--------	1.5	2.61	Solid
10	--------	--------	1.7	2.90	Solid	10	--------	--------	1.7	2.90	Solid
10	--------	--------	2.0	3.33	Solid	10	--------	--------	2.0	3.33	Solid
10	--------	--------	2.5	4.00	Liquid	10	--------	--------	2.5	4.00	Semisolid

DISCUSSION

Manifestly, in the case of both agar and gelatin, strong acid or alkali in the presence of high temperatures is capable of destroying the jellifying power. Everyone who has made culture media according to accepted methods, i. e., sterilized them after the adjustment of reaction, has found that an acidity of 2 to 2.5 per cent normal HCl or a pH concentration of approximately 4 to 3.5 is the limit of solidification of agar. Alkalis in related proportions in the presence of heat have been found to exert a similar action on the jellifying power of agar. Fellers (1917) however, found that this range of jellifying power for 2 per cent agar could be extended to 5 per cent normal HCl or 5 per cent KOH if the acid or alkali were added while the agar was boiling hot and it was not subsequently sterilized. These highly acid or alkaline media were furthermore employed by him (1916) in studies on soil flora, since appropriate quantities could be transferred by means of a sterile pipette to sterile Petri dishes. When one permits the media to cool before adding the acid or alkali as was done in our studies, and as is indicated in Fellers' work, the range of solidification may be extended very much farther. The application of the principles involved herein are believed to make it possible both to simplify the making of media and to improve methods for investigation on the influence of hydrogen ion concentration on microorganisms. Reference to two recent papers, one by Webb (1919) on the influence of reaction on the germination of fungous spores and the other by Fred and Davenport (1918) on the growth of nitrogen assimilating bacteria, will illustrate the possibilities which may come in similar studies from the use of very acid or very alkaline solid media.

In routine work it will be found to be advantageous to flask and sterilize the media in 200 cc. quantities for the reason that the addition of 1 or 2 drops of strong acid or alkali to this quantity will bring about a change in concentration of about pH 0.2. When acid is added to agar in flasks at 50° to 60°C. it may be thoroughly agitated by whirling, 10 cc. portions may be removed for comparison in reaction with the color standards of Clark and Lubs (1917) and when the usual precautions against contamination are observed the material in the flasks may be kept sterile, while the adjustment to the desired pH concentration is being made. The agar may then, before it has had time to solidify, be poured into sterile test tubes or sterile Petri dishes, whereupon it is ready for use. The danger of contamination from this procedure, as judged by experience in making several thousand tubes of media, is no greater than when the tubes are sterilized after the media have been placed in them, as is usually done. In summary, this procedure removes the necessity of sterilization after adjustment of reaction,* eliminates the chances of change of reaction or of other chemical changes which may be hastened by a rise in temperature and does not, within a wide range, destroy the jellifying powers of the agar or gelatin.

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*Experience gained in later studies has led to the adjustment of reaction of *cooled sterile* media and to the avoidance of subsequent heating as a routine practice. Either strong acid or strong alkali or sterile solutions of dilute acid or alkali have been employed. This procedure is by no means novel and has much to commend it. As long ago as 1897, Deeleman (1) corrected his media by adding sterile acid or sterile alkali after the media were sterilized because he noted that titrable acidity is increased by sterilization. Much of the data obtained in these early investigations before accurate methods were elaborated for determining true acidity rather than titrable acidity are now only of historic interest. The necessity of avoiding sterilization subsequent to adjustment of pH is emphasized, however, in a number of recent studies as illustrated by that of Wyeth in 1919 (2).

The temperature of the medium at time of adjustment is another factor whose importance is not generally recognized although its significance, in a manner quite different from that shown in the present paper, was strikingly demonstrated by Clark in 1915 (3). He employed a medium consisting of 10 per cent gelatin, 1 per cent peptone and 0.5 per cent meat extract which, when titrated hot before final sterilization was reported to have a titrable acidity of +10. After sterilization, the acidity should have been greater. Instead, it showed a titrable acidity of +5, with pH=8.5 as the neutral point. At room temperature the titre was +6, at the boiling point with undiluted medium +10, and with 5 cc. diluted to 50 cc. with conductivity water, +19. In conclusion, Clark states that no procedure deserves more severe criticism than hot titrations and that they are irrational since titres thus obtained cannot be translated into hydrogen ion concentrations, in consequence of which a large body of available data is ruined. An increasing mass of data, based on the classic works of Sörensen and Michaelis, must be regarded as proving beyond question that the determining of titrable acidity of media, no matter what its temperature, is an illogical procedure which cannot yield values that measure true acidity and should, therefore, be universally abandoned in bacteriologic technic.

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F. A. W.

CONCLUSION

Agar or gelatin media, if cooled before being made acid or alkaline will jellify at limits far beyond pH concentrations tolerated by microorganisms. They may be manipulated so as to avoid contamination during adjustment of reaction and need not be subsequently sterilized.

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II. Further Studies on Bacterial Blight of Soybean*

It will be recalled that two papers on bacterial blight have appeared during the past year, one from investigations conducted in Wisconsin by Miss Coerper (1) and the other in North Carolina by the junior writer (5). The disease in the case of the former of these accounts, is ascribed to a hitherto undescribed organism, Bacterium glycineum, and in the latter to an organism to which the name Bacterium sojae has been applied. Furthermore, the paper by Miss Coerper appeared in published form, while the junior writer's manuscript was in press, so that opportunity was not afforded for a critical comparative study of the two diseases and of the causal organisms. The foot-note (1.c.p. 120) accompanying this account was prepared by comparison with the published descriptive report, as has been indicated. Through the courtesy and kindness of Miss Coerper and of Dr. L. R. Jones, specimens and cultures have subsequently been received and it is, therefore, the present purpose to record the results of these comparative studies in order to clarify the problem of the identity of these soybean bacterial blight diseases.

DIFFERENCE IN APPEARANCE OF DISEASES

Comparison of type specimens of diseased leaves shows that no differences are present which are sufficiently distinctive to make it possible to separate the two in the field. As previously indicated, however, minor differences, as in the color of the diseased areas and of the dried bacterial exudate, are apparent, which no doubt vary dependent upon weather conditions and the stage of development of the disease.

Even though the disease in North Carolina has been quite prevalent during the present season, it appears to be confined to the leaves and has not been observed also on stems and pods as is the case in Wisconsin. It is doubtful if any means of identification save isolation of the causal organism can be relied upon. Isolations made during the present season from leaves collected in the vicinity of Raleigh, Oxford, and Edenton, N. C., agree with those of previous seasons. Opportunity has not been afforded, therefore, to observe under field conditions, the two leafspot diseases, which as indicated by the cultural studies to follow, must occur in Wisconsin.

ORIGIN OF CULTURES

Five strains of soybean blight, four from Wisconsin and one from North Carolina, have been employed in the cultural studies. Data on those from Wisconsin can best be presented by quoting rather fully Miss Coerper's notes of explanation which accompanied the cultures:

No. 268. Isolated August 29, 1917. Naturally infected leaves were placed in damp chamber over night. Drops of bacterial exudate developed and streaks on potato agar slants were made by direct transfer. One of these isolations proved pathogenic when atomized on soybean leaves. Pathogenicity proved a number of times. Type strain. Brown's peptone media. Employed in all cultural and inoculation work in soybean blight studies.

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*Reprinted from Phytopathology 11: No. 1, pp. 18-24, 1921.

No. 269. Isolated August 31, 1918. Young leaf lesions were placed in 95 per cent alcohol for an instant, then for one minute in 1-1000 solution of bichlorid of mercury and washed through six sterile water blanks. Dilution plates on potato agar were made from macerated lesions. Pathogenicity proved from inoculations in the field in 1918 and in the greenhouse during the winter of 1918-1919. Brown's peptone media.

No. 270. Isolated January 17, 1919, from naturally infected leaf collected in the greenhouse. Surface sterilization was effected as in the case of No. 269 and dilution plates made. Does not brown peptone media. Pathogenicity proven by inoculation of plants in the greenhouse.

No. 211. This is the first strain isolated in the study of the disease, and was the strain used in all cultural and much of the inoculation work in comparison with the type strain No. 268. Isolated September 25, 1915. Does not brown peptone media.

The strain from North Carolina, herein designated No. 19, was isolated June 12, 1919, agrees culturally with a strain isolated during July of the previous year, has several times been proven to be pathogenic and is the type strain of Bacterium sojae.

CULTURAL STUDIES

Method. Since it was early found to be impossible to verify the reaction of the strains toward carbohydrates by use of media prepared according to methods which have previously been employed, other methods which included a more accurate adjustment of initial reaction and a more careful preparation of sugars were adopted. These methods take advantage of recent refinements in methods of adjusting the reaction of culture media colorimetrically and electrometrically to supplant the inaccurate and illogical titration method which employed phenolphthalein as an indicator. They also take into account the well known fact that not only acid or alkali but also heat, can hydrolyze sugars and therefore interfere with the proper preparation of sugar media.*

As is indicated in the preparation of Krumweide's brilliant green agar, sugars in distilled water, and therefore apart from the influence of a complex solvent like culture media with its acid or alkali content, can be sterilized without appreciable hydrolysis and the sterile sugar can then be added to sterile media, using aseptic precautions to prevent the necessity of subsequent sterilization. Accordingly, stock 20 per cent sugar solutions in distilled water were sterilized in the autoclave at 10 pounds for 10 minutes. Stock nutrient agar and bouillon were made as usual, were adjusted to the desired hydrogen ion concentration by comparison with the buffer color standards, were flasked and sterilized in 95 cc. quantities and then set aside until cool.

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*Physiological chemists have long recognized that hydrogen ion concentration and temperature are important factors which influence the decomposition of carbohydrates. In alkaline solution, all of the monosaccharides and some of the disaccharides are unstable. In acid solution the decomposition of monosaccharides proceeds more slowly and is less complete than in alkaline, and in any case, the reaction is hastened by boiling. Further, the di-and polysaccharides are less stable in acid than in alkaline solutions. (See Matthews, A. P. Physiological Chemistry 2d edit. 1915, N. Y.)

The application of these facts is made the basis of an investigation by Mudge (1) who pointed out that maltose and lactose in water solution or in peptone broth seem to be hydrolyzed more by heating for three successive days in an Arnold than by autoclaving at 15 pounds for 15 minutes, while sucrose and raffinose in water solution did not break down.

Advantage has been taken of these facts by a considerable number of bacteriologists, not only in their published investigations but in routine work as well. The preparation of stock solutions of sterile carbohydrates in distilled water will no doubt in time become a generally adopted laboratory practice.

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When 5 cc. of the sugar solution was added to such quantities of media, using aseptic precautions, it contained 1 per cent of the sugar to be investigated. In the case of agar, which had an initial hydrogen ion concentration of pH=7.0-7.2 the sugar was added to cooled but not yet solid media. It was then poured before the agar had set, into sterile test tubes, and was incubated for 48 hours to determine its freedom from contamination.

In making solid indicator media, sterile litmus solutions were prepared and the litmus was added in the same manner as the sugars. After growth had proceeded for a given time, in bouillon cultures containing sugars, which were used to check the results with solid indicator media, tests for acid were made by adding brom cresol purple or phenol red to the cultures and comparing them directly with the standards and with the uninoculated tubes. This last precaution seems to be of significance in the light of findings by Grace and Highberger (3) on variation in uninoculated media.

Litmus agar. Litmus agar in triplicate sets for each strain and for each of the sugars was used as indicator media. Each series included dextrose, saccharose, lactose, maltose and glycerine.

Furthermore, each series was inoculated from the same stock culture and all cultures were incubated at 28 degrees C. with the resultant reaction indicated by the litmus. No differences in strains were apparent on dextrose since the blue had practically disappeared from all tubes by the seventh day and they were reddened throughout. A slight reddening began on all strains on saccharose after three days, and the upper part of the stab was definitely reddened by the seventh day. On each of lactose, maltose, and glycerine, Nos. 268 and 269 were able to bring about a slight change which was first apparent after about a week and the upper portions of the tubes became with age, a reddish brown, whereas, Nos. 211, 270 and 19 produced no acid from these sugars.

Sugar broths. In order to check the changes in reaction in solid media, the several carbon compounds were employed in 2 per cent peptone solutions. The media which had been adjusted to pH=7.2 were inoculated and the changes after seven days were determined by comparison with buffer color standards. These data in terms of pH concentration are presented in the following tabulation:

REACTION TO SUGARS IN PEPTONE BROTH BY SOYBEAN BLIGHT ORGANISMS

Strain	Dextrose	Saccharose	Maltose	Lactose	Glycerine
268	5.4	6.2	6.7	6.5	6.2
269	5.4	6.4	6.4	6.4	6.4
19	6.6	7.0	7.4	7.5	7.6
211	6.4	6.6	7.6	7.4	7.6
270	6.3	6.4	7.6	7.6	7.6

It will be noted that the results are in general accord with those on indicator media in that Nos. 268 and 269 are able to form acid from each of the sugars, whereas Nos. 211, 270 and 19 form acid from dextrose and saccharose alone.

Fermentation tubes. Sterile fermentation tubes were filled under aseptic conditions with sugar broths. The dissolved oxygen gas was not driven off, but it is not apparent that such a procedure is of value since it remains to be proven that heated broths do not again take up oxygen on cooling. The several strains have given similar results with heated and unheated tubed broths. No turbidity developed in the closed arm in seven-day old cultures, thus indicating that the organisms are strictly aerobic. Other organisms may of course react differently, but the dispelling of dissolved oxygen is apparently a factor of less importance than the agitation given fermentation tubes in handling them during examination. Slight agitation may cause apparent growth in the closed arm in the case of all strains so that it becomes easily possible to misinterpret the oxygen relation through the use of fermentation tubes.

Character of colonies. It was only by increasing the percentage of agar above that which the writer was accustomed to use that it was possible to duplicate the appearance of colonies with respect to margin, contour and surface markings shown in Plate XV of Miss Coerper's paper. While it has long been known that the density and viscosity of media exert a profound influence upon the character of the colonies (2), the importance of these factors has been largely overlooked in studies involving the cultural characters of bacteria. In the case of the soybean organisms, the differences in the character of the margins in 1, 2, and 3 per cent nutrient agars which were alike in all other respects are shown in figure 1. It will be seen that the margin of the colony becomes more uneven as the density and viscosity increases. Size, elevation and surface markings are also profoundly modified.

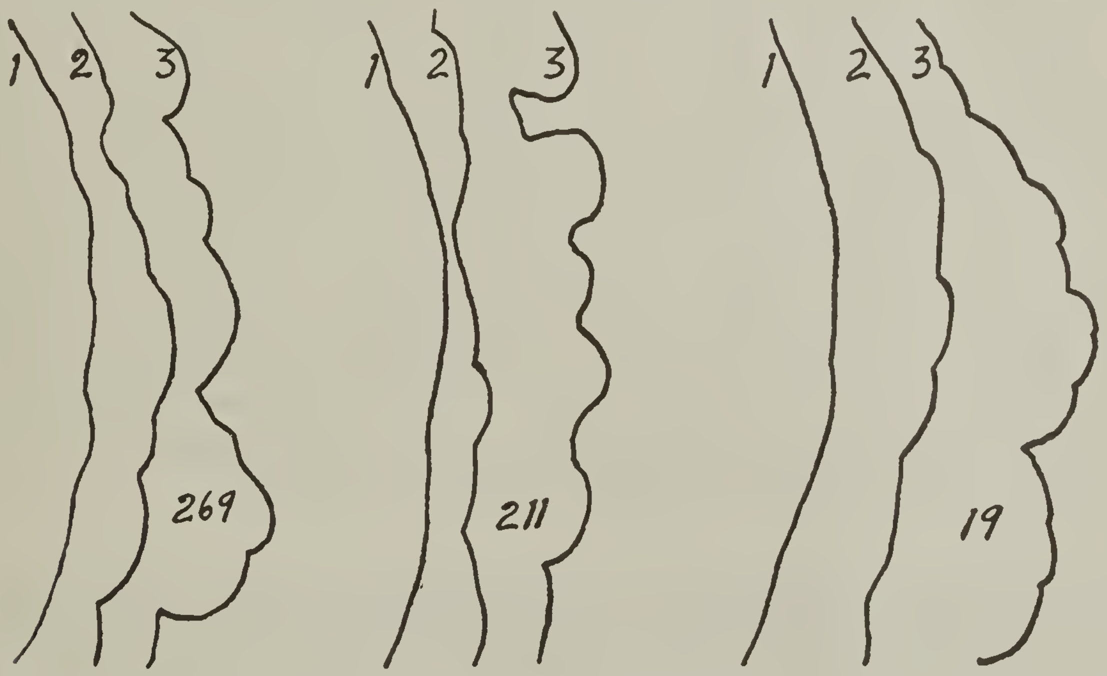

Fig. 1.—Variation in the margin of colonies of Nos. 269, 211 and 19. When grown on 1, 2, and 3 per cent nutrient agar, as seen under the high power.

DISCUSSION

By avoiding the heating of media after the addition of the sugar, one prevents the reaction between the amino groups and the sugar with the resultant production of acidity as occurs during sterilization. Since these strains form acid weakly, their reactions are not evident with media whose initial reaction, as determined by titration with phenolphthalein is approximately pH=8.5. When, however, the initial reaction closely approximates absolute neutrality, as determined colorimetrically, small increases in acidity consequent on growth are detectable. This is shown particularly in the case of No. 19, which with litmus agar adjusted to pH=8.0-8.5 gives no evidence of its ability to form acid with saccharose. A similar condition has been reported by Jones (4) for a strain of Pneumococcus which when inoculated into a medium whose initial reaction was pH 7.0 grew poorly and developed a final concentration of only pH 6.2, whereas in a medium whose initial reaction was pH 7.6 growth was abundant and a concentration of pH 5.4 was developed.

It has been possible to verify previously published accounts of the morphology and cultural characters of the soybean bacterial blight organisms in all respects except in the reaction toward the several sugars. Nos. 268 and 269, which in Miss Coerper's discussion on variations of strains (1 l. c. p. 189) are called Strain A, are regarded as identical and are the type of Bacterium glycineum. They have the same reaction toward dextrose, saccharose, lactose, maltose, and glycerine, from the first two of which they form acid rather strongly, but from the others, in small amounts and slowly. They furthermore, consistently cause a browning of the media. Nos. 211 and 270, which are called Strain E, are like No. 19, the type of B. sojae. They are able to form acid from dextrose and saccharose alone and agree in being non-pigment forming.*

SUMMARY

A comparison has been made of bacterial blight of soybean as described from Wisconsin and from North Carolina. The diseases differ somewhat in appearance, but these differences are of minor importance and it is doubtful if one could differentiate them with certainty in the field. By the use of certain easily operable refinements which consist essentially in adjustment of initial reaction to neutrality and in reducing the hydrolyzation of sugars to negligible quantities, it is determined that the several strains of bacteria pathogenic to soybeans represent two distinct species, Bacterium glycineum and B. sojae. They differ principally in that the former produces brown pigment with certain media and forms acid from dextrose, saccharose, lactose, maltose and glycerine, whereas the latter is non-pigment forming and forms acid from the first two of these sugars only. Both organisms have been isolated in Wisconsin and proven to be pathogenic, and B. sojae alone has been found to be associated with soybean blight in North Carolina.

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*In fermentation studies conducted during the summer of 1921, additional evidence was secured of differences of specific rank between Nos. 19 and 268. In bouillon consisting of 1 per cent peptone, 0.3 per cent beef extract, 0.5 per cent NaCl, and 1 per cent of either mannite, galactose, or dextrine, Bacterium glycineum was able to ferment both mannite and galactose, but not dextrine, with the formation of acid. No acid was formed from any of these carbohydrates by B. sojae. The initial reaction was adjusted to pH 7.2 in all cultures. By the seventh day, with B glycineum, the reaction with mannite had changed to pH 6.4, with galactose, pH 5.2 and with dextrine pH 7.8. In the case of B. sojae, 7.6, 7.8 and 7.8 represented the corresponding H ion concentrations respectively on the seventh day. *F. A. W.*

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III. Tolerance to Acids of Certain Bacterial Plant Pathogenes*

INTRODUCTION

Cultural studies of plant pathogenic bacteria are generally believed to be incomplete unless they include a determination of the lethal or limiting hydrogen and hydroxyl ion concentration. Recent investigations on forms pathogenic to man have demonstrated, by the employment of refined methods, the importance and value of hydrogen ion determinations. Similar studies by plant pathologists, employing methods of precision in measuring acidity and alkalinity, in place of the meaningless titrimetric methods, would no doubt, show that H-ion concentration is of significance in the culture of plant pathogenic forms. Certainly in such an important problem as the possible correlation between tolerance to acid and immunity or resistance no progress has been made with methods in vogue and no convincing body of evidence has thus far been adduced. Neither will such data be forthcoming until a better understanding is had of some of the fundamental factors underlying the inhibitory or antiseptic action of acids.

Further, the influence of the hydrogen ion on inhibition of growth and on the processes of metabolism in bacteria is generally believed both to be specific and an easily explicable matter. It become obvious, however, that such is not the case, and that much additional experimentation is necessary before an adequate explanation can be offered for the phenomena which may be noted if one cultivates several species of bacteria on media which differ only in the kind of acids employed in making the pH adjustment.

In the present investigation, attention has been directed to the nature of the acid and to the nature of the medium as factors in the problem of the influence of pH. There has arisen, outside the field of plant pathogenic bacteriology, a considerable body of literature dealing with inhibition and antiseptic action. Some of these investigations, as will be discussed later, point out the interesting fact of the failure of acids of the same pH concentration to inhibit cell multiplication, the cause of which has not been satisfactorily explained. This observation has been confirmed in the present study with plant pathogenic forms and in addition, differences in growth in liquid and solid media are noted which further indicate the complexity of the problem of inhibition.

METHOD

The organisms employed were Bacillus carotovorus, Bacterium campestre, Bacterium angulatum, Bacterium tabacum, Bacterium glycineum and Bacterium sojae. They were cultivated in bouillon, consisting of 1 per cent Armour's peptone, 0.3 per cent Liebig's beef extract, and 0.5 per cent NaCl and in nutrient agar prepared by adding 2 per cent agar to the bouillon. Two per cent of agar and 1 per cent of peptone were arbitrarily employed after some preliminary experimentation upon the effect of variation of the agar content and of the peptone between 1 and 3 per cent. As is well known, both of these substances, except in the case of pure agar, modify the "buffer" action, and consequently the amount of acid necessary to secure the desired pH, but within the range of 1 to 3 per cent, they appear to be without effect on inhibition of growth.

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*Reprinted from Phytopath. 11:244-250. 1921.

After dissolving the materials, the media were neutralized, flasked in 200 cc. quantities and sterilized. As soon as they had cooled to about 60°C. commercially pure concentrated acid of the appropriate kind was added, using aseptic precautions, to obtain the desired pH values. The pH values were determined colorimetrically by comparison with buffer color standards. These buffer solutions were checked electrometrically and were found to be less than their assigned value by .03-.06 pH. After the medium had been adjusted to the desired H-ion concentration, it was poured into sterile test tubes. Such a procedure as demonstrated in a previous paper (1) is of especial value in the preparation of solid media since it removes the necessity of sterilization after adjustment of reaction and thus does not modify the acidity nor destroy the jellifying power. All tubed media were incubated for 48 hours before inoculation to determine their freedom from contamination.

RESULTS

Growth in bouillon, as shown in Table 1, was determined by the ability of the several organisms to cloud the media. In the case of abundant clouding, the sign + is employed, no visible growth is indicated by—and a slight clouding by—+.

Manifestly the H-ion concentration alone does not determine the limit of tolerance of these organisms since one would then expect irrespective of the acid, cellular multiplication to be checked at the same point. The several organisms are seen to be more tolerant to malic than to any other of the acids and to be inhibited most by acetic. At a pH value of 4.6 when malic acid was employed, Bacterium tabacum made a slight growth, whereas with acetic acid, the limit lies between 5.8-6.0. In the case of Bacillus carotovorus, inhibition by these two acids occupies a much more restricted range since growth was checked between 4.6 and 4.8 by malic acid and 5.3 is the corresponding limit with acetic. Bacterium campestre is considerably more intolerant to formic acid than is Bacillus carotovorus since 5.5 and 4.8 represent the respective limits.

Differences in vigor of growth on the agar slants are indicated by the same signs and the data are summarized in Table 2.

The most striking fact exhibited by growth on solid media as contrasted with liquid media is the ability of the several organisms to withstand a greater H-ion concentration when grown on the former. In general, this difference appears to vary from 0.2-0.4 pH. Just as in liquid media, all organisms are less tolerant to acetic than to the other acids. Bacillus carotovorus appears to be considerably less sensitive to acetic acid, however, than any of the other organisms since its limit lies between pH 4.8-5.0 and the others between pH 5.5-5.8.

DISCUSSION

The fact that acids of different hydrogen ion concentration can exert a similar influence in checking bacterial growth has previously been noted, as has been indicated. Winslow and Lochridge (2), using B. coli and B. typhosus, found that the disinfecting power of acids was proportional to the concentration of the hydrogen ion, but noted in the case of acetic acid an effect greater than that due to the H-ion concentration. In explanation, they suggest that this is due to the undissociated acid remaining in the solution.

TABLE 1.—TOLERANCE OF VARIOUS ORGANISMS TO ACID IN BOUILLON

Name of Organism	pH 4.6							pH 4.8							pH 5.0							pH 5.3							pH 5.5		pH 5.8	pH 6.0
	Hydrochloric	Sulphuric	Acetic	Citric	Tartaric	Malic	Formic	Hydrochloric	Sulphuric	Acetic	Citric	Tartaric	Malic	Formic	Hydrochloric	Sulphuric	Acetic	Citric	Tartaric	Malic	Formic	Hydrochloric	Sulphuric	Acetic	Citric	Tartaric	Malic	Formic	Acetic	Formic	Acetic	Acetic
Bacterium campestre	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	+	+	+	—	+	+	—	+	+	+	—	—	+	+	+
Bacillus carotovorus	—	—	—	—	—	—	—	—	+	—	—+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	*+	—	+	+
Bacterium glycineum	—	—	—	—	—	—	—	—	—	—	—	—+	+	—	+	—	—	+	+	+	+	+	+	—	+	+	+	+	—	—	—	+
Bacterium sojae	—	—	—	—	—	—	—	—	—	—	—+	—	+	—	+	—	—	+	—+	+	+	+	+	—	+	+	+	+	—	—	—	—+
Bacterium angulatum	—	—	—	—	—	—	—	—	—+	—	—+	—+	+	—	+	+	—	+	+	+	+	+	+	—	+	+	+	+	—	—	—	+
Bacterium tabacum	—	—	—	—	—	—+	—	—	+	—	—	—+	+	—	+	+	—	+	+	+	+	+	+	—	+	+	+	+	—	—	—	+

*Through error the original paper indicates no growth at pH 5.5 with acetic acid.

TABLE 2.—TOLERANCE OF VARIOUS ORGANISMS TO ACIDS IN AGAR

Name of Organism	pH 4.4			pH 4.6			pH 4.8				pH 5.0				pH 5.4	pH 5.5	pH 5.6	pH 5.8
	Hydrochloric	Sulphuric	Oxalic	Hydrochloric	Sulphuric	Oxalic	Hydrochloric	Sulphuric	Oxalic	Acetic	Hydrochloric	Sulphuric	Oxalic	Acetic	Acetic	Acetic	Acetic	Acetic
Bacterium campestre	—	—	—	—	—	—	—+	+	+	—	+	+	+	—	—	—+	+	+
Bacillus carotovorus	—	—+	—	+	+	+	+	+	+	—	+	+	+	+	+	—+	—+	+
Bacterium glycineum	—	—	—	—	+	—	—+	+	—	—	+	+	+	—	—	—	—	+
Bacterium sojae	—	—	—	—	—	—	+	+	—	—	+	+	+	—	—	—	—	+
Bacterium angulatum	—	—	—	—	—	—	+	+	—	—	+	+	+	—	—	—	—	+
Bacterium tabacum	—	—	—	—	—	—	—+	+	—	—	+	+	+	—	—	—	—+	+

More recently, Cohen and Clark (3) from cultural studies on B. coli pointed out that the factor or factors which obscure the effect of the H-ion are quite unknown. They noted that this organism grew well at pH 5.0 when adjustment was made with HCl, but at pH 5.45 with acetic acid the same organism declined in numbers. Other organisms exhibited the same difference in conduct with reference to these two acids. They explain this as probably due to a synergic effect of the free acetate radicle upon the disinfecting power of the hydrogen ion, a view in accord with those who hold that the anions and undissociated molecules play an important role in the kinetics of disinfection by accelerating the action of the hydrogen ion. They also suggest the probability that this difference corresponds to a difference in permeability of the organism to mineral and organic acid.

Were one in possession of full knowledge of the unknown factor involved in the foregoing observations, he could no doubt explain what is believed to be a comparable condition reported by Svanberg (4). He found that Streptococcus lactis (Bacterium lactis acidi) in milk is capable of producing an acidity which corresponds to the value pH=4. When, however, lactic acid is added to milk, growth is checked at pH=4.4-4.7 and acetic acid at pH=4.8-5.1.

The plant pathogenes used in the present investigation appear to respond differently toward the various acids just as did the several organisms employed in these several studies. This may be taken to indicate that the various acids exert a specific effect. Granted that the effect is specific, it is not clear how one could employ this fact in explaining the general shift of the limit of tolerance of 0.2-0.4 pH as occurs between bouillon and nutrient agar. It is felt, however, that this observation should be reported at this time on its own merit, subject to verification, and with the admission that no adequate explanation of the cause is known to us. It is believed that the fact of a greater pH tolerance on solid media is of significance in studies which attempt to correlate the acidity of juices of certain plants with tolerance in culture of forms parasitic on these same plants.

In conclusion, the present studies appear to be in accord with several others previously discussed in showing that the activity of the hydrogen ions alone is not responsible for the inhibitory effects which have been observed. This is opposed by the conclusions of a number of investigations, among which are those of Norton and Hsu (5), who inclined to the view that the disinfecting power of an acid is approximately proportional to the hydrogen ion concentration. Whatever may be the ultimate solution of this problem, it is apparent that further developments will depend upon the devising of methods which will show what are the dissociation products of the various acids in such complex solvents as bouillon or agar and whether or not these dissociation products in the presence of the H-ions modify the permeability of the bacterial cell wall.*

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*The complexity of the problem of the influence exerted by the hydrogen ion on bacterial inhibition is further demonstrated by the voluminous literature in the field involving a study of antiseptics and by several bacteriological papers which either were not available when this manuscript was submitted or appeared while it was in press. It is entirely outside the scope of the present paper to summarize the work on the H-ion and its relation to inhibition and antisepsis. A brief statement regarding a few papers will suffice to show this relationship and to guide the reader to a proper appreciation of this problem. Friedenthal (1) in a study of the comparative antiseptic power of various substances and ionic groups noted that no ion is of such powerful antiseptic virtue as H+, one gram of which was computed to be capable of maintaining sterility in 30,000,000 liters of culture medium. Paul and Krönig (2) noted that the toxicity of metallic salts for Bacillus anthracis and Staphylococcus aureus depends not only on the cation but on the anion and undissociated molecules as well. The same idea is maintained in later work by Paul and his associates in two papers which appeared in 1910 (3 & 4). In addition, it is pointed out that the hydrogen ions of weak organic acids appear to be catalyzed by the anions and that neutral salts, when added to inorganic acids, are capable of increasing the toxicity of these acids.

In a comparative study of the standardization of disinfectants by the Rideal-Walker and the Hygienic Laboratory methods, Wright (5) found that the discrepancies noted by himself and others were due in part to variation in the hydrogen ion concentrations of different batches of media. In addition, the pH value of the Rideal-Walker broth was approximately 7.65, while that of the Hygienic Laboratory was 5.55; media so different could not be expected to give comparable results.

The several bacteriological investigations to which reference was made are based upon a "physiological constant" postulated by Michaelis and Marcora in 1912 (6). They showed that B. coli ceased the production of acid in a lactose bouillon at a concentration of 1×10^{-5}N, which point they considered to be a physiological constant for this organism. Several factors, however, serve to modify the final reaction at which activity ceases. Clark (7) found that B. coli from bovine feces brought a 1 per cent peptone plus 1 per cent dextrose to pH=4.26, while with 1 per cent lactose only pH=4.56 was reached. He also noted distinct differences in the final reaction with the same organism in different media, i. e., the greater the buffer effect of the medium, the lower the final H-ion concentration attained. The results of studies by Wolf and Harris (8) show that the final reaction varies dependent on two other factors, namely: the reaction of the medium before inoculation and the kind of acid used. With Welches' bacillus in 2 per cent peptone plus 2 per cent glucose broth, when adjustment is made with HCl to an initial reaction of pH 6.77 growth ceases when sufficient acid is produced to give the medium a reaction of pH 4.59. When the initial reaction was pH 4.86 a final reaction of pH 4.34 was attained. Initial reactions between these two extremes give final reactions which take up orderly positions between pH 4.59 and pH 4.34, showing that there is a definite relation between initial and final reaction. The results with B. sporogenes agreed in showing the influence of initial hydrogen ion concentration upon final hydrogen ion concentration. Their studies show for each acid a "critical pH," which is the mean of the most acid value of pH which permits growth and the least acid value which inhibits growth. In the case of Welches' bacillus, the "critical pH" with hydrochloric acid was 4.78; with lactic, 4.68; with formic, 4.91; with malic, 4.66; with acetic, 4.88; with butyric, 4.99; and with succinic, 4.88. Bacillus sporogenes was less tolerant to these acids as shown by the "critical pH" 4.87 with hydrochloric; 4.87 with lactic; 4.91 with formic; 5.08 with acetic; and 4.96 with butyric.

Wyeth (9) in experimentation with B. coli extended the observations of Wolf and Harris (8) and concluded in agreement with them, that the initial hydrogen ion concentration of the medium and the nature of the acid used to produce this initial reaction are both factors which modify the final reaction by a culture. In addition, he found that in 2 per cent peptone adjusted with hydrochloric acid a "critical pH" for B. coli of 4.27 was obtained, but when phthalate was substituted for peptone, as a buffer material, this value became pH 4.55; with lactic acid the corresponding "critical pH" values are 4.47 and 4.73 and with acetic, 4.68 and 4.83 in peptone and phthalate respectively.

Foster (10) has recently noted another interesting condition which modifies the range of tolerance. In 1 per cent glucose broth the limits of growth of Streptococcus hemolyticus were pH 6.35 and pH 8.50. If, however, 5 per cent horse serum was added to this broth, these limits were extended to pH 5.90 and pH 9.25.

Manifestly, then, there is no definite hydrogen ion concentration at which growth ceases or which can be tolerated by bacteria which is not modified by one or more of the following factors:

1. Kind of organism
2. Kind of acid used in adjustment or the medium
3. Initial reaction of the medium
4. Kind of buffer material
5. Concentration of buffer material
6. Kind of fermentable carbohydrate
7. Concentration of fermentable carbohydrate
8. Presence of substances like serum which stimulate growth and fermentation probably through their content of food accessory substances.
9. Physical state of medium, i. e. whether solid or liquid.

A number of other factors, some of which are more or less well known, such as strain differences, age of the parent culture, "salt" effects, temperature, autotoxins, etc., have been noted to exert an influence.

In summary, therefore, studies on tolerance to or inhibition by H or OH ions are of little or no value unless accompanied by definitely specified conditions which must include a statement of the exact composition of the experimental media.

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SUMMARY

Cultural studies have been made on the tolerance to acids of various plant pathogenic bacteria, including the cabbage black rot organism, one commonly causing decay of root crops, two causing leaf spots of soybean and two causing tobacco leaf spots.

Different acids of the same H-ion concentration are not able to exert the same influence in inhibiting cell multiplication. Acetic is more toxic than any other of the acids employed, an observation which accords with that of others.

A greater pH concentration in agar than in bouillon is required to inhibit growth, this difference ranging between 0.2 and 0.4 pH with the same organism and the same acid.

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IV. Thermal Death Points of Some Bacterial Plant Pathogenes in Relation to Reaction of the Medium

Those who have interested themselves in a study of the reaction of culture media know of its profound influence upon the morphology of bacteria, their viability, enzyme formation, pigment production, and rate of fermentation and putrefaction as recorded in scores of instances. Quite a few investigations, too, but all of which so far as is known, have been conducted outside the field of plant pathology, have noted that hydrogen ion concentration affects also the thermal death point. It is the present purpose, therefore, to report on studies of several organisms pathogenic to plants in order to show the influence of this factor upon their thermal death points.

METHODS

Subcultures of the organisms employed in the previous study (1) were used in these tests. Bacillus carotovorus, Bacterium campestre and B. glycineum, the latter from type material, were obtained through the courtesy of the Department of Plant Pathology of the University of Wisconsin. The others, Bacterium tabacum, B. sojae and B. angulatum were isolated in this laboratory, the first two of which are subcultures from type strains. Cultures of these several organisms were grown for twenty-four hours in a medium consisting of 2 per cent agar, made up with a watery extract from boiled potatoes, plus 2 per cent dextrose. Transfers were made from these vigorously growing cultures to bouillon prepared from 1 per cent Armour's peptone, 0.3 per cent Liebig's beef extract and 0.5 per cent NaCl in distilled water. All of the bouillon was made in the same lot, was then divided into ten portions, flasked, cooled, adjusted to the desired pH concentration with HCl or NaOH, tubed, and then incubated for forty-eight hours to determine its sterility. Triplicate sets of each organism in each of the ten concentrations were exposed for ten minutes to the temperature conditions of the water bath. This bath had a capacity of about eight gallons, and could, with vigorous stirring, be maintained within a range of two-tenths degree of the desired temperature. All exposures were made as soon as possible after the transfers had been made. Further, all were incubated for four days at 20-25°C. after exposure and death was determined by their inability to cloud the media.

RESULTS

The summary in Table 1 shows the differences obtained. In the case of visible clouding of the media the sign + is employed and no visible growth is indicated by —.

Under the conditions of this experiment as modified by the effect of reaction, the thermal death point of B. sojae lies between 44-48°C. of B. glycineum, 43-48°C. of B campestre 45-51°C. of B. angulatum 45-51°C. of B. tabacum 46-51°C. and of B. carotovorus 47-51°C. In general it will be seen that the greatest sensitivity to higher temperatures appears to be manifested at extreme reactions.

TABLE 1.—INFLUENCE ON THERMAL DEATH POINT OF VARYING pH CONCENTRATIONS

Organisms	Temperature, Degrees C.	pH Concentrations									
		5.4	5.8	6.2	6.6	7.0	7.4	7.8	8.3	8.6	9.0
Bacterium sojae	44	+	+	+	+	+	+	+	+	+	+
	45	—	+	+	+	+	+	+	+	+	—
	46	—	+	+	+	+	+	+	+	—	—
	47	—	—	—	+	+	+	+	—	—	—
	48	—	—	—	—	—	—	—	—	—	—
Bacterium glycineum	43	+	+	+	+	+	+	+	+	+	+
	44	+	+	+	+	+	+	+	+	—	—
	45	+	+	+	+	+	+	+	+	—	—
	46	—	+	+	+	+	+	+	—	—	—
	47	—	—	—	+	+	+	+	—	—	—
	48	—	—	—	—	—	—	—	—	—	—
Bacterium campestre	45	+	+	+	+	+	+	+	+	+	+
	46	+	+	+	+	+	+	+	+	—	—
	47	+	+	+	+	+	+	+	+	—	—
	48	+	+	+	+	+	+	+	+	—	—
	49	+	+	+	+	+	+	+	—	—	—
	50	+	+	+	+	+	+	+	—	—	—
	51	—	—	—	—	—	—	—	—	—	—
Bacterium angulatum	45	+	+	+	+	+	+	+	+	+	+
	46	+	+	+	+	+	+	+	+	—	—
	47	+	+	+	+	+	+	+	+	—	—
	48	+	+	+	+	+	+	+	+	—	—
	49	+	+	+	+	+	+	+	—	—	—
	50	+	+	+	+	+	+	+	—	—	—
	51	—	—	—	—	—	—	—	—	—	—
Bacterium tabacum	46	+	+	+	+	+	+	+	+	+	+
	47	+	+	+	+	+	+	+	+	—	—
	48	—	+	+	+	+	+	+	+	—	—
	49	—	+	+	+	+	+	+	—	—	—
	50	—	+	+	+	+	+	+	—	—	—
	51	—	—	—	—	—	—	—	—	—	—
Bacillus carotovorus	47	+	+	+	+	+	+	+	+	+	+
	48	+	+	+	+	+	+	+	+	—	—
	49	—	+	+	+	+	+	+	—	—	—
	50	—	—	—	+	+	+	—	—	—	—
	51	—	—	—	—	—	—	—	—	—	—

DISCUSSION

It may be of interest in connection with the data which have been presented above to call attention to points of contact with the work of others. Pasteur (2) recognized that the expression "thermal death point" has no significance when considered apart from the agents or processes involved in pasteurization as attested by his statement that "It is easy to show that these differences in temperature which are required to secure organic liquids from ultimate change depend exclusively upon the state of the liquids, their nature and above all upon conditions which affect their neutrality whether towards acids or bases."

It is beside the point to present here any exhaustive review of the literature from Pasteur's time to the recent studies. Suffice it to say that acidity and alkalinity have, in consequence of more exact methods for their measurement, come to be better known as agents which influence the thermal relations of bacteria and molds. Clark (3) pointed out the influence of hydrogen ion concentration on the so-called thermal death point of B. coli in a paper presented to the Society of American Bacteriologists, in 1914, which contained the statement that "in cellular destruction temperature is to be regarded as an accelerating *condition* and that among the active *agents* concerned, the concentration of the hydrogen ions of a medium may be of great significance."

Later studies by Cohen and Clark (4) show that B. coli can survive a temperature of 45°C. for different lengths of time, depending upon the two factors, pH concentration and kind of acid used. The most marked reduction in number of viable organisms was found by them to be at the acid extremes. This effect of reaction on cellular destruction is entirely in accord with that indicated by the several plant pathogenes at both the H and OH extremes.

One would also expect as indicated by the results with B. coli and by the previous studies on tolerance to acids (1) that the same pH concentrations secured through the use of different acids would modify the thermal death points shown in Table 1 of these plant parasites. This specific effect of acids would, of course, be most evident as the limits of tolerance are approached and indicates the close interaction and interrelation between nature of the acid, pH concentration and temperature.

This relationship of reaction and thermal death point obtains also among spore bearing bacteria as concluded by Bigelow and Esty (5) and Weiss (6) who found that as the H and OH ion concentration is increased, the time required for the complete destruction of the spores is decreased. The latter author found in addition that sodium chlorid lowers the thermal resistance of B. botulinus and that the rate of lowering increases rapidly as the concentration of this salt is increased. In the light of investigations by Paul, Birstein and Reuss (7) this lowering of thermal resistance through the addition of NaCl is correlated with increased toxicity of the acids resulting from the reaction between the salt and acids.

SUMMARY

A study has been made of the relation of reaction to the thermal death point of Bacillus carotovorus, Bacterium glycineum, B. sojae, B. campestre, B. angulatum and B. tabacum. The organisms were exposed to the different temperatures in plain bouillon adjusted with HCl or NaOH. Variations of 4° to 6°C. are noted between pH 5.4 and pH 9.0, points within the H and OH ion limits of tolerance.

These data, obtained from studies with bacterial parasites of plants, confirm the idea that the concentration of hydrogen ions is an agent of great significance in cellular destruction at high temperatures.

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V. The Fermentative Activity of Some Plant Pathogenic Bacteria in Relation to Hydrogen Ion Concentration

The classification of bacteria is based, in part, upon their ability to ferment carbohydrates. The reliability of such fermentation tests for diagnostic purposes, moreover, has frequently been brought into question in physiological investigations. This doubt has been largely removed, during the past few years, however, through a more adequate knowledge of the physico-chemical laws which govern fermentation and other microbial activities as well. The classical works of Sörensen (19 and 20) and Michaelis (17), basic to the development of methods for the measurement of hydrogen ion concentration and in consequence to the numerous researches which have demonstrated its importance, have contributed more than any other factor to this proper understanding. Singularly, in researches with bacterial plant pathogenes, the influence of the hydrogen ion has been accorded only scant attention and has never been emphasized although a very considerable interest exists in this group of organisms as manifested by the number of published investigations. The character of these investigations, furthermore appears to be extensive and descriptive rather than intensive and analytical, when contrasted with medicobacteriological studies and no methods especially adapted to work with plant parasites have been developed. Reasons for this are not far to be sought since most of our knowledge of bacterial diseases of plants, a field containing a wealth of new or little known problems, has been acquired from studies during the past ten years and since in training phytopathologists too little emphasis has been placed upon a fundamental knowledge of plant physiology and chemistry. The future alone will disclose to what extent anything of value is to be gained from studies in plant bacteriology comparable to those in medico-bacteriology on such problems as the carbohydrate and nitrogen metabolism, or any other phase of what may be included under bacterial dietetics, as their biochemistry or as the influence of hydrogen ion concentration in so far as it may affect rate of cell multiplication, production of pigment, rate of fermentation or putrefaction, immunity of the host, time of infection, etc.

The present inquiry into the relation of hydrogen ion concentration to the fermentative activity of some organisms from plant tissues is based upon recent researches in sanitary and medical bacteriology. Comparison of results and methods will, therefore, be freely made in establishing points of similarity or difference. As might be anticipated, there are many points of similarity between these two groups of organisms. It might be surmised, too, that a number of fundamental differences exist between species in either group which could not be brought to light by the employment of any stereotyped technic. That such is the case and that a knowledge of the physiology of one species should lead one to generalize with caution regarding the physiology of another becomes apparent in the course of the studies to be herein reported.

METHODS AND TECHNIC

Organisms. Two organisms causing leafspot diseases of tobacco, Bacterium tabacum and B. angulatum, two causing leafspots of soybean, B. glycineum and B. sojae. one vascular parasite of cruciferous plants, B. campestre. and a storage rot organism of vegetables and root crops, Bacillus carotovorus. have been employed in these studies. The cultures were secured from the sources noted in a previous paper (4), and have been maintained in stock on potato agar.

Culture media. Plain bouillon, consisting of 1 per cent Armour's peptone, 0.3 per cent Liebig's beef extract and 0.5 per cent NaCl in distilled water. served as the basis of the media employed throughout the work. Twenty per cent solutions, in distilled water, of the carbohydrates were sterilized at 10 pounds for ten minutes. Addition of carbohydrate to bouillon was made with aseptic precautions. The carbohydrates were of the purity known as "bacto sugars" prepared by the Digestive Ferments Company. Prepared media were always incubated for twenty-four to forty-eight hours previous to inoculation to insure sterility. Strong solutions of HCl and NaOH have been employed in adjustment of reaction. No media were heated after adjustment of reaction or after the addition of carbohydrate.

pH Determinations. Clark and Lubs (9) standard buffer solutions which differed by increments of 0.2 pH were prepared. These were checked electrometrically and were found to vary from their assigned value by a few points at most in hundredths place. Tubes containing the medium to be tested were compared colorimetrically with these standards containing the appropriate indicators. By the use of the method of superposition in a comparator block, it was possible to make readings correct to within 0.1 pH. A considerable number of cultures of each organism was employed in each series. Changes in reaction were followed by readings at definite consecutive intervals. Each consecutive reading was made with a different culture.

EXPERIMENTAL

It seemed advisable in the beginning to obtain information as to the optimum initial pH concentration for growth in bouillon and to the character and rate of change of reaction in this medium, and in broths of each of the two component materials, beef extract and peptone, before giving consideration to the fermentations in carbohydrate bouillons.

Experiment I—The Influence of Hydrogen Ion Concentration on the Rate of Cell Multiplication in Bouillon

Cultures adjusted to the reactions shown in Table 1 were grown in flasks of 250 cc. capacity filled to one-half their capacities for the initial volume of bouillon. To avoid the lag phase, transfers from stock agar were grown for 24 hours in tubes of bouillon of the same composition with a pH value of 7.2 and inoculations were then made from these cultures. A temperature of 23°-25°C. was maintained. The changes in growth were followed by removing at two hour intervals, under aseptic conditions, a one cc. sample after having rotated the flasks briskly to thoroughly mix the contents. This sample was diluted and plated in duplicate in potato dextrose agar, composed of 2 per cent shredded agar made up with watery extract from boiled potatoes plus 2 per cent dextrose. These plates were counted after an incubation period of four days.

Table 1.—Influence of Initial pH Concentration of Bouillon on Rate of Cell Multiplication

Name of Organism	Age of Culture Hours	Number of Bacteria per cc.							
		pH 5.4	pH 5.8	pH 6.4	pH 7.0	pH 7.4	pH 7.8	pH 8.4	pH 9.0
Bacterium glycineum	0	5000	5000	4000	----------	3000	2000	2000	1000
	2	6000	13000	8000	----------	5000	3000	1000	1000
	4	8000	22000	17000	----------	8000	5000	2000	1000
	6	12000	35000	38000	----------	14000	8000	2000	1000
	8	15000	50000	80000	----------	28000	11000	3000	2000
Bacterium sojae	0	1000	8000	3000	2000	--------	2000	1000	2000
	2	1000	8000	5000	3000	--------	4000	1000	3000
	4	1000	9000	9000	6000	--------	8000	2000	3000
	6	1000	13000	18000	18000	--------	17000	5000	5000
	8	2000	30000	37000	42000	--------	30000	10000	6000
Bacterium campestre	0	2000	8000	8000	12000	--------	10000	9000	6000
	2	2000	8000	13000	16000	--------	14000	9000	5000
	4	3000	13000	18000	23000	--------	19000	13000	6000
	6	4000	21000	22000	31000	--------	30000	16000	8000
	8	4000	28000	30000	60000	--------	60000	28000	12000
Bacterium angulatum	0	13000	35000	45000	74000	--------	34000	73000	--------
	2	14000	45000	53000	82000	--------	54000	75000	--------
	4	20000	85000	65000	216000	--------	109000	90000	--------
	6	23000	180000	191000	560000	--------	240000	150000	--------
	8	30000	300000	320000	1200000	--------	530000	250000	--------

Name of Organism	Age of Culture Hours	pH 5.2	pH 5.7	pH 6.2	pH 7.0	pH 8.0	pH 8.5	pH 9.0
Bacterium tabacum	0	18000	38800	26400	31400	38900	34800	18000
	2	17500	72000	55000	72500	66500	70000	18200
	4	20000	130000	120000	160000	120000	150000	19000
	6	21000	270000	250000	340000	230000	700000	20000
	8	22000	740000	650000	112000	540000	1100000	21000
	10	22000	1220000	1400000	2700000	1200000	2000000	22000

Name of Organism	Age of Culture Hours	pH 5.4	pH 5.8	pH 6.4	pH 7.2	pH 7.6	pH 8.0	pH 8.5
Bacillus carotovorus	0	6000	14000	16000	7000	17000	15000	10000
	2	8000	20000	28000	11000	28000	20000	14000
	4	12000	42000	50000	22000	49000	48000	26000
	6	20000	99000	130000	40000	112000	90000	47000
	8	33000	180000	350000	95000	170000	200000	90000

Due to the large experimental error involved in the dilution plate method and in counting the plates, the numbers of bacteria in these tabulations are at best approximations. Changes in reaction in any of the media during the short period covered by the experiment were insignificant and in no way contributed to modify the rate of growth as will be apparent in studies to be reported in subsequent pages. In general, it will be seen that the different organisms react similarly to the influence of initial reaction. There is a broad zone of pH within which the rate of cell multiplication is fairly uniform. This is entirely in accord with the findings of Cohen and Clark (10) with B. coli. At the borders of this zone, near the critical acid and alkali limits, however, there is a marked effect. Were one to plot a curve showing these points, using the average generations per hour as ordinates against pH as abscissae, this curve would be essentially a straight line until the borders of the zone are reached in which region, between the maximum values which permit growth and the minimum values which inhibit growth, there is an abrupt slope to the curve. In the case of the several plant pathogenes, the character of the curve therefore is quite like that of B. coli (10), in showing that the chief effect of reaction seems to be confined to the limiting H and OH extremes. These borders vary with the different organisms, as has been demonstrated in a previous paper (23.)

One other point of special interest is indicated also in Table 1 but can best be shown by a summary such as is presented in Table 2.

TABLE 2.—GENERATIONS PER HOUR WITHIN DEFINITE pH ZONES

Organism	pH Range	Average Generations per Hour
Bacterium glycineum	5.4 — 8.4	.36
Bacterium sojae	5.4 — 8.4	.37
Bacterium campestre	5.4 — 8.4	.23
Bacterium angulatum	5.4 — 8.4	.37
Bacterium tabacum	5.2 — 8.5	.47
Bacillus carotovorus	5.4 — 8.5	.44

The average number of generations per hour, of these several organisms, within their range of growth is seen to be small when contrasted with such organisms as B. coli and B. aerogenes whose rate of growth was found by Cohen and Clark (10) to be 2.5 and 2.7, respectively, during their period of logarithmic increase. It should be remembered in this connection that these last named organisms can produce vigorous fermentation, whereas these plant parasites, as will be shown later, are all relatively slow acting. Whether or not there is a parallelism between generation time and rate of fermentation remains to be determined. Cullen and Chesney (11) have shown a close relationship between the rate of cell multiplication and acid production by pneumococcus in plain broth. Their data show that a rise in the growth curve slightly precedes the rise in acidity. Quite different results however were secured by Jones (14) with pneumococcus in glucose broth. His curves show a sharp rise in number of cells at the fourth or fifth hour with a delay of seven hours in the onset of the period of acid production.

Experiment II—The Nature of the Change in Reaction in Beef-extract Broth

In these tests, a 0.3 Liebig's beef extract broth with an initial reaction of pH 7.2 was employed. The daily changes in reaction with each of the organisms is shown in the following table:

TABLE 3.—FINAL pH IN 0.3 BEEF EXTRACT BROTH

Organism	Age of Cultures in Days and pH Concentration					
	Days					
	1	2	3	4	5	6
	pH	pH	pH	pH	pH	pH
Bacterium tabacum	7.2	7.4	7.4	7.4	7.6	7.8
Bacterium angulatum	7.2	7.4	7.4	7.6	7.6	7.8
Bacillus carotovorus	7.2	7.2	7.2	7.4	7.6	7.8
Bacterium glycineum	7.2	7.2	7.2	7.4	7.6	7.6
Bacterium sojae	7.2	7.2	7.2	7.4	7.6	7.6
Bacterium campestre	7.2	7.2	7.2	7.4	7.4	7.4

This medium supports meagre growth in the case of all of these organisms so that little significance perhaps can be attached to this experiment. All cultures, however, appear to become progressively more alkaline.

Experiment III—The Changes in Reaction in Peptone Broth

One per cent Armour's peptone broth with an initial pH of 7.2 was used. In Table 4 are shown the pH concentrations at given intervals.

TABLE 4.—CHANGES IN pH CONCENTRATION IN 1 PER CENT ARMOUR'S PEPTONE BROTH

Organism	Age of Cultures in Days and pH Concentration					
	Days					
	1	2	4	6	8	11
	pH	pH	pH	pH	pH	pH
Bacterium tabacum	7.2	7.4	7.6	8.2	8.4	8.4
Bacterium angulatum	7.2	7.4	7.6	8.2	8.4	8.4
Bacillus carotovorus	7.0	7.0	7.0	7.4	7.4	7.6
Bacterium glycineum	7.2	7.2	7.6	8.0	8.4	8.4
Bacterium sojae	7.2	7.2	7.4	7.8	8.2	8.4
Bacterium campestre	7.2	7.4	7.8	8.0	8.0	8.4

The only point of interest in this table is the slight but definite increase in acidity in cultures of B. carotovorus. This increase is followed by a reversal of reaction with a subsequent gradual increase in alkalinity. These changes are more marked in plain bouillon as shown in Table 5 which follows.

A similar increase in hydrogen ion concentration in sugar free broth has been noted in the case of a number of organisms, including Micrococcus aureus

(15) and Streptococcus hemolyticus (12). The investigations involving these two organisms explain this phenomenon on the basis of a *selective* action by the organism on the carbohydrate* radical of the protein molecule. After the carbohydrate is exhausted there is a reversal of reaction since the organism must then attack the protein for its supply of structural and fuel materials.

Experiment IV—Changes in Hydrogen Ion Concentration in Plain Bouillon

After determining the nature of the changes in reaction in beef extract broth and peptone broth, it seemed logical to employ plain bouillon in similar tests. Further, since this plain bouillon was to serve as a stock to which the carbohydrates were to be added in the fermentation tests to be next reported, it seemed desirable to know of the changes in reaction produced in plain bouillon alone. Accordingly, each of the organisms was cultured with the results summarized in Table 5.

TABLE 5.—CHANGES IN REACTION IN PLAIN BOUILLON, 1 PER CENT PEPTONE, 0.3 BEEF EXTRACT, 0.5 NaCl. INITIAL pH 7.2

Organism	Age of Cultures in Days and pH Concentrations						
	Days						
	1	2	4	6	8	10	12
	pH	pH	pH	pH	pH	pH	pH
Bacterium tabacum	7.2	7.2	7.4	7.6	8.2	8.2	8.4
Bacterium angulatum	7.2	7.2	7.4	7.6	8.0	8.2	8.4
Bacillus carotovorus	6.8	6.6	6.8	7.4	8.0	8.2	8.4
Bacterium glycineum	7.2	7.2	7.4	7.6	8.0	8.2	8.4
Bacterium sojae	7.2	7.2	7.4	7.4	7.8	8.2	8.4
Bacterium campestre	7.2	7.2	7.4	7.8	8.0	8.2	8.2

All except B. carotovorus are seen to cause a progressive increase in alkalinity. This change proceeds until the point of inhibition by OH ions is approximated. Whether or not other factors than concentration of hydroxyl ions influence inhibition is not known since no effort has been made to determine the presence or absence of such products of metabolism as are usually designated "autotoxins." In the case of B. carotovorus, there is at first, as pointed out in Table 4, an increase in acidity which is followed by a reversal of reaction and a gradual change toward the point of the OH limit of tolerance.

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*The occurrence of protein substances which contain a carbohydrate group has been known for a long time. The nature of this carbohydrate which can be split off by acid and which may amount to as much as 35 per cent in some proteins has been explained chiefly by the investigations of Friedrich Muller. (See Hammarsten and Hedin, Textbook of Physiological Chemistry, 7th edition, 1914, p. 84). This carbohydrate is always an amino-sugar, and generally glucosamine. All proteins do not contain a carbohydrate group and future investigators must decide whether the carbohydrate groups belong positively to the protein complex or whether they are united with the proteins only as impurities.

Experiment V—The Influence of Varying Concentrations of Dextrose, Saccharose, Lactose and Glycerine Upon the Fermentative Activity

Since acids are elaborated through the fermentation of carbohydrates by bacteria, one would expect on purely theoretical grounds, that the greater the concentration of carbohydrates the more potential acid. Under cultural conditions, however, definite optimum carbohydrate concentrations have been found to obtain in the case of the several species of bacteria which have been investigated, in order to permit of the production of their characteristic final pH concentrations. Browne (7) found that 1 per cent of lactose for B. coli is optimum, that less titrable acid is produced in broth containing less than 1 per cent of lactose and that over 1 per cent of this sugar resulted in no increase. Sekiguchi (17) noted that 0.5-2.0 per cent of glucose is necessary for the maximum production of acid by streptococci. Avery and Cullen (2) found that 0.4 per cent of glucose is sufficient to permit the greatest production of acid with pneumococcus. Fred, Peterson and Davenport (13) in studies with xylose fermenting organisms found that 2 per cent of this sugar gave the maximum acidity. Streptococcus hemolyticus, according to Foster (12) requires between 0.2 and 0.3 per cent glucose dependent upon initial reaction in order to attain its maximum pH value.

The influence of the factor initial reaction upon final reaction, resultant upon fermentation, is furthermore demonstrated in studies by Wolf and Harris (21) with Bacillus Welchii and B. sporogenes, and by Wyeth (25) with B. coli. Various phases of another factor, buffer materials and buffer salts, and their influence upon the concentration of carbohydrate necessary if the organism in question is to produce its characteristic final pH concentration, have been investigated by Clark (8), Kligler (16), Bronfenbrenner and Schlesinger (6) and Foster (12). These studies have established the fact that buffer materials and buffer salts modify the rate of fermentation, the level of acidity, and concentration of sugar necessary in obtaining this final reaction. In the present studies which have been summarized in the Tables 6-9, media of the same initial reaction and buffer content with a variation only in carbohydrate concentration have been employed so that these factors may be disregarded in the data as presented.

TABLE 6.—INFLUENCE OF VARYING CONCENTRATIONS OF DEXTROSE IN PLAIN BOUILLON UPON THE FINAL pH CONCENTRATIONS. INITIAL pH 7.2

Organisms	Concentration of Dextrose	Age of Cultures in Days and pH Concentrations							
		Days							
		1	2	3	4	5	7	9	11
	Per Cent	pH	pH	pH	pH	pH	pH	pH	pH
Bacterium tabacum	0.1	7.0	7.0	7.0	7.2	7.4	7.8	8.4	8.4
	0.3	7.0	7.0	6.8	7.2	7.4	7.6	8.2	8.4
	0.6	7.0	6.8	6.4	6.4	6.8	7.2	7.8	8.4
	1.0	7.0	6.2	6.0	6.2	6.2	6.4	7.4	8.0
	2.0	7.0	6.0	5.6	5.4	5.2	5.0	5.0	5.0
Bacterium angulatum	0.1	7.0	7.0	7.0	7.0	7.4	7.8	8.2	8.4
	0.3	7.0	7.0	6.8	7.0	7.4	7.6	8.4	8.4
	0.6	7.0	6.8	6.4	6.4	6.6	7.0	7.6	8.2
	1.0	7.0	6.2	6.2	6.4	6.4	6.8	7.4	8.0
	2.0	7.0	6.0	5.6	5.4	5.2	5.2	5.0	5.0
Bacillus carotovorus	0.1	5.6	5.4	5.4	5.6	6.2	7.4	8.2	8.4
	0.3	5.6	5.2	5.2	5.0	5.0	5.0	5.0	5.0
	0.6	5.4	5.2	5.0	5.0	5.0	5.0	5.0	5.0
	1.0	5.4	5.2	5.2	5.0	5.0	5.0	5.0	5.0
	2.0	5.4	5.2	5.2	5.0	5.0	5.0	5.0	5.0
Bacterium glycineum	0.1	7.0	7.0	6.6	7.2	7.6	8.0	8.2	8.4
	0.3	7.0	7.0	7.0	6.8	7.0	7.4	7.8	8.2
	0.6	7.0	6.8	6.8	6.4	6.8	7.0	7.6	8.0
	1.0	7.0	6.8	6.6	6.6	6.4	6.6	7.0	7.4
	2.0	7.0	6.8	6.4	6.2	5.8	5.2	5.0	5.0
Bacterium sojae	0.1	7.0	7.0	7.0	7.2	7.6	8.0	8.2	8.4
	0.3	7.0	7.0	7.0	7.2	7.4	7.6	8.0	8.2
	0.6	7.2	6.8	6.8	6.8	7.0	7.2	7.6	8.0
	1.0	7.0	6.8	6.8	6.6	7.0	7.2	7.4	7.8
	2.0	7.0	6.8	6.4	6.4	6.2	6.0	5.6	5.4
Bacterium campestre	0.1	7.2	7.2	7.2	7.2	7.4	7.6	7.8	7.8
	0.3	7.2	7.2	7.2	7.2	7.4	7.4	7.8	7.8
	0.6	7.2	7.2	7.2	7.4	7.4	7.6	7.8	7.8
	1.0	7.2	7.2	7.2	7.2	7.4	7.6	7.6	8.0
	2.0	7.2	7.2	7.2	7.2	7.4	7.4	7.4	7.6

TABLE 7.—INFLUENCE OF VARYING CONCENTRATIONS OF SACCHAROSE IN PLAIN BOUILLON UPON THE FINAL pH CONCENTRATIONS. INITIAL pH 7.2

Organism	Concentration of Saccharose	Age of Cultures in Days and pH Concentrations								
		Days								
		1	2	3	4	5	7	9	11	13
	Per Cent	pH	pH	pH	pH	pH	pH	pH	pH	pH
Bacterium tabacum	0.1	7.2	7.2	7.2	7.4	7.6	7.8	8.2	8.4	8.4
	0.3	7.2	7.2	7.2	7.4	7.6	8.0	8.4	8.4	8.4
	0.6	7.0	6.8	6.8	6,4	6.2	6.6	7.4	8.0	8.4
	1.0	7.0	6.6	6.4	6.4	6.2	6.4	7.0	7.6	8.2
	2.0	6.8	6.4	6.0	5.6	5.6	5.2	5.2	5.0	5.0
Bacterium angulatum	0.1	7.2	7.2	7.2	7.6	7.8	8.0	8.4	8.4	8.4
	0.3	7.2	7.2	7.2	7.4	7.6	8.0	8.2	8.4	8.4
	0.6	7.2	7.0	7.0	7.0	7.4	7.6	8.2	8.4	8.4
	1.0	7.0	6.6	6.4	6.3	6.2	6.4	7.0	7.6	8.0
	2.0	6.8	6.4	6.0	5.6	5.5	5.4	5.2	5.2	5.2
Bacillus carotovorus	0.1	6.4	6.2	6.2	6.4	6.5	6.6	6.8	7.4	7.6
	0.3	5.4	5.4	5.3	5.2	5.2	5.2	5.2	5.2	5.2
	0.6	5.4	5.2	5.2	5.1	5.0	5.0	5.0	5.0	5.0
	1.0	5.3	5.2	5.1	5.1	5.0	5.0	5.0	5.0	5.0
	2.0	5.2	5.2	5.1	5.1	5.0	5.0	5.0	5.0	5.0
Bacterium glycineum	0.1	7.2	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.4
	0.3	7.2	7.2	7.4	7.6	7.6	8.0	8.2	8.4	8.4
	0.6	7.2	7.0	6.9	6.8	7.0	7.3	7.6	8.0	8.2
	1.0	7.2	7.0	6.8	6.6	6.2	6.4	7.0	7.2	7.4
	2.0	7.0	6.6	5.8	5.2	5.2	5.1	5.0	5.0	5.0
Bacterium sojae	0.1	7.2	7.2	7.4	7.6	7.7	8.0	8.2	8.4	8.4
	0.3	7.2	7.2	7.4	7.6	7.6	8.0	8.2	8.3	8.4
	0.6	7.2	7.0	7.0	6.9	6.8	7.2	7.4	7.8	8.2
	1.0	7.2	7.0	6.8	6.6	6.4	6.2	6.8	7.2	7.6
	2.0	7.0	6.6	6.2	5.8	5.6	5.4	5.2	5.1	5.0
Bacterium campestre	0.1	7.2	7.2	7.3	7.4	7.4	7.6	7.6	7.7	7.8
	0.3	7.2	7.2	7.3	7.4	7.4	7.5	7.6	7.6	7.8
	0.6	7.2	7.3	7.4	7.4	7.4	7.5	7.6	7.8	7.8
	1.0	7.2	7.2	7.3	7.4	7.4	7.6	7.6	7.8	7.8
	2.0	7.2	7.2	7.2	7.2	7.2	7.3	7.3	7.4	7.6

TABLE 8.—INFLUENCE OF VARYING CONCENTRATIONS OF LACTOSE IN PLAIN BOUILLON UPON THE FINAL pH CONCENTRATIONS. INITIAL pH 7.4

Organisms	Concentration of Lactose	Age of Cultures in Days and pH Concentrations							
		Days							
		1	2	3	5	7	9	11	13
	Per Cent	pH	pH	pH	pH	pH	pH	pH	pH
Bacterium tabacum	0.1	7.4	7.4	7.6	7.8	8.0	8.2	8.4	8.4
	0.3	7.4	7.4	7.6	7.8	8.2	8.4	8.4	8.4
	0.6	7.4	7.4	7.6	7.8	8.2	8.4	8.4	8.4
	1.0	7.4	7.5	7.6	7.8	8.0	8.4	8.4	8.4
	2.0	7.4	7.6	7.6	7.8	8.4	8.4	8.4	8.4
Bacterium angulatum	0.1	7.4	7.4	7.6	7.8	8.4	8.4	8.4	8.4
	0.3	7.4	7.4	7.6	7.8	8.4	8.4	8.4	8.4
	0.6	7.4	7.6	7.8	8.4	8.4	8.4	8.4	8.4
	1.0	7.4	7.6	7.8	8.0	8.2	8.4	8.4	8.4
	2.0	7.4	7.6	7.7	7.8	8.1	8.4	8.4	8.4
Bacillus carotovorus	0.1	5.8	5.6	5.6	6.4	7.4	7.8	8.2	8.4
	0.3	5.6	5.4	5.3	5.2	5.2	5.2	5.2	5.2
	0.6	5.4	5.3	5.2	5.2	5.2	5.2	5.2	5.2
	1.0	5.4	5.3	5.2	5.1	5.0	5.0	5.0	5.0
	2.0	5.3	5.2	5.2	5.1	5.0	5.0	5.0	5.0
Bacterium glycineum	0.1	7.4	7.4	7.5	7.6	7.8	8.2	8.4	8.4
	0.3	7.4	7.4	7.5	7.6	7.8	8.2	8.4	8.4
	0.6	7.4	7.4	7.6	7.6	7.8	8.0	8.4	8.4
	1.0	7.4	7.4	7.2	7.0	7.4	7.8	8.0	8.2
	2.0	7.4	7.2	7.0	6.8	7.2	7.6	8.0	8.2
Bacterium sojae	0.1	7.4	7.4	7.6	7.8	8.0	8.2	8.4	8.4
	0.3	7.4	7.4	7.6	7.8	8.0	8.2	8.4	8.4
	0.6	7.4	7.4	7.6	7.8	8.0	8.2	8.4	8.4
	1.0	7.4	7.4	7.6	7.8	8.0	8.2	8.4	8.4
	2.0	7.4	7.4	7.6	8.0	8.2	8.3	8.4	8.4
Bacterium campestre	0.1	7.4	7.4	7.4	7.4	7.5	7.6	7.8	8.0
	0.3	7.4	7.4	7.4	7.4	7.5	7.6	7.8	8.0
	0.6	7.4	7.4	7.4	7.4	7.8	8.0	8.2	8.2
	1.0	7.4	7.4	7.4	7.4	7.8	8.0	8.4	8.4
	2.0	7.4	7.4	7.4	7.4	7.8	8.0	8.4	8.4

TABLE 9.—INFLUENCE OF VARYING CONCENTRATIONS OF GLLYCERINE IN PLAIN BOUILLON UPON THE FINAL pH CONCENTRATIONS. INITIAL pH 7.2

Organisms	Concentration of Glycerine	Age of Cultures in Days and pH Concentrations					
		Days					
		1	2	4	6	8	10
	Per Cent	pH	pH	pH	pH	pH	pH
Bacterium tabacum	0.1	7.2	7.4	7.6	7.8	8.2	8.4
	0.3	7.2	7.4	7.6	7.8	8.0	8.4
	0.6	7.2	7.4	7.6	7.7	7.8	8.0
	1.0	7.2	7.2	7.4	7.6	7.8	8.0
	2.0	7.2	7.2	7.4	7.6	7.8	8.0
Bacterium angulatum	0.1	7.2	7.4	7.6	7.8	8.0	8.2
	0.3	7.2	7.4	7.6	8.0	8.2	8.4
	0.6	7.2	7.2	7.4	7.6	7.8	8.0
	1.0	7.2	7.2	7.3	7.4	7.6	7.8
	2.0	7.2	7.2	7.2	7.4	7.6	8.0
Bacillus carotovorus	0.1	7.2	7.0	7.0	6.8	7.0	7.2
	0.3	7.2	7.0	6.8	6.6	6.4	6.8
	0.6	7.2	7.0	6.8	6.6	6.4	7.0
	1.0	7.2	6.8	6.4	6.8	7.2	7.4
	2.0	7.2	6.8	6.4	6.2	6.0	5.6
Bacterium glycineum	0.1	7.2	7.2	7.4	7.6	7.7	7.8
	0.3	7.2	7.2	7.0	7.0	7.2	7.6
	0.6	7.2	7.0	6.8	7.0	7.2	7.4
	1.0	7.2	7.0	6.8	6.4	6.8	7.2
	2.0	7.2	7.0	6.8	6.4	6.8	7.2
Bacterium sojae	0.1	7.2	7.2	7.4	7.6	7.8	8.4
	0.3	7.2	7.2	7.4	7.5	7.6	7.8
	0.6	7.2	7.2	7.2	7.4	7.4	7.6
	1.0	7.2	7.2	7.2	7.4	7.4	7.6
	2.0	7.2	7.2	7.2	7.3	7.4	7.6
Bacterium campestre	0.1	7.2	7.2	7.4	7.6	8.0	8.2
	0.3	7.2	7.2	7.4	7.6	8.0	8.4
	0.6	7.2	7.2	7.5	7.6	8.2	8.4
	1.0	7.2	7.2	7.6	7.6	8.0	8.4
	2.0	7.2	7.2	7.6	7.8	8.0	8.2

It will be seen in the first place that B. carotovorus and B. glycineum are able to ferment all of these sugars with the formation of acid. The former attacks all vigorously so that the characteristic final reaction is attained within a few days. The latter ferments dextrose and saccharose quite vigorously but requires a longer period of time to reach this ultimate point, and there is a reversal of reaction with lactose and glycerine. None of these sugars are attacked by B. campestre. B. tabacum, B. angulatum and B. sojae are able to ferment only dextrose and saccharose.

Further, very marked differences exist in rates of fermentation by these forms as shown when B. carotovorus is contrasted with any of the others. In general, the former will reach its final reaction within three days, whereas five to nine days are required for the others. Nearly all of this change with B. carotovorus will have occurred within the first 24-hour period, and in this respect this organism is like the pneumococci, streptococci and the colon group. The type of fermentation produced by the others, which is apparently uncommon among animal pathogenic forms, results in a slow, progressive increase in acidity. Again, B. carotovorus is seen to require a minimum carbohydrate concentration between 0.1 and 0.3 per cent and amounts up to 2 per cent have no further effect upon the level of the final pH concentration. The minimum concentration of sugars for the others lies between 1.0 and 2.0 per cent under the same experimental conditions. At a concentration of 1 per cent of dextrose or saccharose, there is seen with these other forms, to be a reversal of reaction, beginning on about the fifth day, by which time the reaction will have changed from an initial pH of 7.2-7.4 to pH 6.0-6.4. Forty-eight to seventy-two hours with 2 per cent dextrose or saccharose is sufficient time for an increase in acidity to this same point with these same organisms. This reversal of reaction is a phenomenon which has recently been investigated by Ayers and Rupp (3) and is due as they explain, not to the formation of ammonia, but to the fermentation of organic acids to carbonates. The simultaneous fermentation of the sugar and of the organic acids, as pointed out by them, does not decrease the value of sugar fermentations for diagnostic purposes, but should lead to a broader conception of the problem. In the present studies, the reversal of reaction with 1 per cent and less of sugar and not with 2 per cent may be taken to indicate that for these organisms it is associated as a casual condition with insufficient fermentable sugar to permit the attainment of the characteristic level of acidity. The carbon metabolism of B. carotovorus is manifestly quite different from that of B. tabacum, B. angulatum, B. glycineum and B. sojae. Whether plant pathogenes will be found by subsequent investigations to conform to a number of distinct types, such as have been noted by Berman and Rettger (4), remains to be determined. They found that B. coli and Proteus vulgaris, which represent one type, ferment glucose rapidly with the formation of large amounts of acid. Such appears to be the case with B. carotovorus. B. subtilis, representative of another type, they found to attack glucose slowly, as is the situation with B. tabacum, B. angulatum, B. sojae and B. glycineum. B. subtilis utilized both carbohydrate and protein at the same time, a condition contrary to the common belief, that when both utilizable carbohydrate and protein are present in a medium, there is a "sparing action" of the sugar. B. cloacae, a third type, formed very little acid since the products of fermentation of glucose and lactose were largely carbon dioxide and hydrogen. It appears unlikely that B. campestre is of this type, since there was no evidence of fermentation of any of the sugars. Analysis of sugar content at definite intervals would, no doubt, serve as the best means of determining whether or not a decrease was occurring and whether the products of fermentation were being utilized as rapidly as they were being formed.

Attention might well be directed, at this point, to the fact that B. tabacum and B. angulatum are indistinguishable on the basis of their ability to ferment the four carbohydrates employed in the fermentation tests of the Descriptive Chart. The original description (24) of the former is in error as regards its ability to ferment glycerine and lactose, due largely to faulty preparation of these sugars. The organisms are, however, not identical. Aside from the fact that they produce diseases which are widely different in appearance, and that they differ in certain morphological features, such as size and flagellation, they are separable on the basis of their fermentative activity, since B. angulatum does not form acid from mannite and galactose as does B. tabacum. Little use has been made, up to the present time, in phytopathological studies, of the rare sugars. Their value, will no doubt, be better appreciated and more emphasized proportional to their use in future investigations.

Another fact of interest which appeared in these fermentation tests is the relation between carbohydrate concentration and pigment production. In plain bouillon, or bouillon plus 1 per cent or less of carbohydrate, B. glycineum develops brown pigment. With 2 per cent of these various sugars, there is an entire failure of the formation of brown pigment. The proximate cause of this inhibition of pigment production, as is generally accepted in investigations with animal pathogenes, is the concentration of hydrogen ions resultant from an optimum supply of fermentable carbohydrate.

Experiment VI—The Influence of Buffer Upon Reaction in Bouillon Containing Varying Concentrations of Dextrose

The bouillon used in this series contained 0.3 per cent Liebig's beef extract, 0.5 per cent NaCl and either 2 or 4 per cent Armour's peptone. The determinations of pH were made at intervals of 48 hours with the results shown in Table 10.

TABLE 10.—INFLUENCE OF DIFFERENT AMOUNTS OF PEPTONES UPON pH CONCENTRATION IN VARYING CONCENTRATIONS OF DEXTROSE. INITIAL pH 7.2

Organism	Concentration of Dextrose	Age of Cultures in Days and pH Concentrations in 2 and 4 Per Cent Peptone									
		Days									
		1		3		5		7		9	
		Peptone		Peptone		Peptone		Peptone		Peptone	
		2%	4%	2%	4%	2%	4%	2%	4%	2%	4%
	Per Cent	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
Bacterium tabacum	0.1	7.2	7.2	7.0	7.2	7.4	7.6	8.0	8.0	8.4	8.4
	0.3	7.2	7.2	6.8	6.8	7.0	7.4	7.4	7.8	8.0	8.4
	0.6	7.2	7.2	6.4	6.4	6.4	6.4	6.8	6.8	7.2	7.4
	1.0	7.0	7.2	6.4	6.4	6.0	5.8	5.8	6.2	6.6	6.6
	2.0	7.0	7.0	6.4	6.4	5.4	5.4	5.2	5.2	5.0	5.0
Bacterium angulatum	0.1	7.2	7.2	7.2	7.2	7.6	7.8	8.0	8.2	8.4	8.4
	0.3	7.2	7.2	7.0	6.8	7.2	7.4	7.4	7.8	7.8	8.4
	0.6	7.2	7.2	6.4	6.6	7.2	7.2	7.4	7.6	7.8	8.0
	1.0	7.2	7.2	6.8	6.2	6.6	6.6	6.8	6.6	7.2	6.8
	2.0	7.2	7.2	6.4	6.4	5.6	6.2	5.4	6.4	5.2	6.6
Bacillus carotovorus	0.1	6.8	6.8	6.6	6.6	6.6	7.0	7.4	7.4	7.6	7.6
	0.3	5.6	5.8	5.4	5.6	5.4	5.6	5.2	5.4	5.2	5.4
	0.6	5.6	5.6	5.4	5.4	5.4	5.4	5.2	5.2	5.2	5.2
	1.0	5.4	5.6	5.2	5.4	5.2	5.4	5.1	5.2	5.0	5.2
	2.0	5.4	5.6	5.2	5.4	5.2	5.2	5.1	5.2	5.0	5.0
Bacterium glycineum	0.1	7.2	7.2	7.0	7.0	7.4	7.4	8.0	8.0	8.2	8.2
	0.3	7.2	7.2	7.0	6.8	7.6	7.4	8.0	8.2	8.4	8.4
	0.6	7.2	7.0	7.0	7.0	7.0	7.6	7.6	8.0	8.0	8.4
	1.0	6.8	7.0	6.2	6.6	6.0	7.4	6.4	7.6	6.8	7.8
	2.0	6.8	7.0	5.6	5.8	5.4	5.4	5.2	5.2	5.0	5.0
Bacterium sojae	0.1	7.2	7.2	7.2	7.0	7.6	7.6	8.0	8.0	8.4	8.4
	0.3	7.2	7.2	7.0	7.0	7.4	7.4	7.8	8.0	8.4	8.4
	0.6	7.2	7.0	7.0	7.0	7.4	7.6	7.8	7.8	8.2	8.4
	1.0	6.8	7.0	6.2	6.6	6.0	7.4	6.6	7.8	7.0	8.0
	2.0	7.0	7.0	6.2	6.4	5.4	6.2	5.2	6.0	5.2	5.6
Bacterium campestre	0.1	7.2	7.2	7.2	7.2	7.2	7.4	7.4	7.6	7.8	8.0
	0.3	7.2	7.2	7.2	7.2	7.2	7.2	7.6	7.6	8.0	8.0
	0.6	7.2	7.2	7.2	7.4	7.2	7.4	7.6	7.6	8.0	8.0
	1.0	7.2	7.2	7.2	7.2	7.2	7.2	7.6	7.6	8.0	8.0
	2.0	7.2	7.2	7.2	7.2	7.4	7.4	7.6	7.6	7.8	8.0

When the data in Table 10 are contrasted with those in Table 6, it will be seen that they differ mainly in the fact that the greater concentrations of peptone tend to decrease the rate of change in reaction. This modifying influence exerted by buffer materials is entirely in accord with previously recorded observation on Streptococcus hemolyticus, B. coli, and other members of the colon-typhoid-dysentery group.

Experiment VII—The Relation of Initial Hydrogen Ion Concentration to The Final Level of Acidity Produced by Bacillus Carotovorus, Bacterium Glycineum and B. Sojae in 2 Per Cent Saccharose Bouillon

The saccharose bouillon used in this experiment was adjusted to the four pH values, 5.5, 6.2, 7.2, and 8.0 before inoculation with the several organisms. The cultures were incubated at 28°C. and the reaction was determined at 24-hour intervals with the results expressed in Table 11.

TABLE 11.—RELATION OF INITIAL TO FINAL pH CONCENTRATION IN 2 PER CENT SACCHAROSE BOUILLON

Organism	Initial pH	Age in Days and pH Concentration						
		Days						
		1	2	3	4	5	6	7
		pH	pH	pH	pH	pH	pH	pH
Bacterium glycineum	5.5	5.5	5.4	5.2	5.1	5.1	5.1	5.1
	6.2	6.2	5.5	5.3	5.2	5.2	5.2	5.2
	7.2	7.2	6.4	5.6	5.4	5.4	5.4	5.4
	8.0	8.0	7.4	6.8	6.6	6.2	6.0	6.0
Bacterium sojae	5.5	5.5	5.5	5.3	5.3	5.2	5.2	5.2
	6.2	6.2	6.2	5.8	5.5	5.4	5.4	5.4
	7.2	7.2	7.2	6.8	6.2	6.0	5.8	5.8
	8.0	8.0	7.8	7.4	7.3	7.2	7.0	6.8
Bacillus carotovorus	5.5	5.2	5.2	5.2	5.1	5.1	5.1	5.1
	6.2	5.4	5.2	5.2	5.2	5.2	5.2	5.2
	7.2	5.4	5.2	5.2	5.2	5.2	5.2	5.2
	8.0	5.4	5.4	5.4	5.4	5.4	5.4	5.4

A maximum period of lag following transplantation occurred in all cultures with an initial of pH 5.5. By the end of four days, B. carotovorus had reached its final reaction which differed by 0.3 pH between the initial extremes. B. glycineum and B. sojae had not completed their activity within seven days, but it is clearly evident that a curve of their reaction resultants would not be a line parallel to the abscissa. Just as in the case of B. Welchii and B. sporogenes as shown by Wolf and Harris (21) and B. coli as shown by Wyeth (25), there is a definite relationship between the initial and the final pH. The highest final levels are attained by cultures with the highest initials and the lowest finals by those with lowest initials. It appears probable also that the reaction resultants reached from any point between the two initial extremes, pH 5.5 and pH 8.0, would fall in a straight line passing between the two extremes of final reaction, i. e., pH 5.1 and pH 5.4 in the case of B. carotovorus.

Experiment VIII—Changes in Reaction as Influenced by Mechanical Agitation

Recent studies by Bonazzi (5) have shown that more vigorous growth and greater physiological activity by Nitrosococcus may be induced by mechanical stirring of the culture solutions. He does not enter into any speculation as to the cause aside from mentioning the possibility that shaking may provide better aeration or assist in the removal of by-products unfavorable to growth. Allen (1) employing this fact found that this type of treatment is beneficial also in cultures of Azotobacter. Their agitating device was so constructed as to hold the culture flasks in a horizontal position and revolve them with a uniform, slow motion. The present series, which is summarized in Table 12, was based upon the results of Bonazzi and Allen. Flasks containing 150 cc. of bouillon of the appropriate carbohydrate concentration were prepared and inoculated with B. glycineum. The initial reaction of the dextrose broths was pH 7.2, and they were maintained at a temperature of 20°-25°C., while the initial reaction of the saccharose broths was pH 7.8 and they were incubated at 28°C. An initial volume of bouillon of 150 cc. in 250 cc. flasks was employed. At twenty-four hour intervals, 5 cc. quantities were withdrawn with aseptic precautions from each flask, for the pH determinations. The flasks which were agitated were attached to a platform which was devised to be used with the Leeds and Northrup shaking apparatus. This machine, which is a part of their apparatus for the electrometric measurement of hydrogen ion concentration, imparted a rocking motion to the flasks. The check culture flasks were of course placed on a shelf where the solutions could remain quiet.

Table 12.—Influence of Agitation of Cultures of Bacterium Glycineum in Dextrose and Saccharose Bouillon on Changes in Reaction

Concentration of Carbohydrate	Age in Days and pH Concentration									
	Days									
	1		2		3		4		5	
	Agitated	Quiet	Agitated	Quiet	Agitated	Quiet	Agitated	Quiet	Agitated	Quiet
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
Per cent of dextrose:										
1.0	6.6	6.6	6.2	6.4	6.0	6.6	5.6	7.0	5.2	7.4
2.0	6.2	6.6	5.6	6.2	5.4	5.8	5.2	5.6	5.0	5.2
Per cent of saccharose:										
1.0	7.4	7.8	7.2	7.6	7.0	7.4	7.4	7.0	7.6	6.8
2.0	7.4	7.6	7.2	7.2	6.2	7.0	5.8	6.6	5.8	6.4

B. glycineum, it should be remembered, is of the type of organism which tends to form a surface pellicle for which reason, it was selected for this experiment. The most striking feature which it exhibits when agitated is the increased rate of growth as evidenced by the clouding of the broth. This condition obtains with both dextrose and saccharose bouillon. This increased rate of growth, in the agitated cultures, is correlated with increased acidity as would be expected. An intensification of activity, therefore of B. glycineum just as with Nitrosococcus and Azotobacter results from agitation.

Another situation for which no explanation can be offered at this time appears in the flasks containing 1 per cent of dextrose. Those which remain undisturbed undergo a reversal of reaction, such as is shown also in Table 6 from which we concluded that 1 per cent dextrose in plain bouillon was insufficient to permit of the development of the characteristic final reaction. When cultures of the same dextrose content are agitated there is seen to be a progressive increase in acidity just as occurs with 2 per cent of this sugar or of saccharose. In the case of 1 per cent saccharose, however, when agitated and also when quiet, there is a reversal of reaction, beginning on the sixth or seventh day.

If one contrasts, furthermore, the daily pH concentrations in this table with those in Table 7, the influence of initial reaction considered in Experiment VII will be apparent.

SUMMARY

Consideration is given, in this study, to the fermentative activity of Bacillus carotovorus, Bacterium tabacum, B. angulatum, B. glycineum, B. sojae and B. campestre, as influenced by initial reaction, concentration and kind of carbohydrate, concentration of buffer material, and mechanical agitation.

All are found to grow at a fairly uniform rate over a wide range of initial reaction. As this reaction approximates pH 5.0 and pH 9.0 as extremes, however, a slight change in pH produces a marked effect upon cell multiplication.

The rate of growth for these forms in terms of generations per hour varies between 0.25 and 0.5, which is considerably less than for forms from animal bodies.

In plain broth cultures, there is a progressive increase in alkalinity with all organisms except B. carotovorus. This form shows at first an increase in acidity, which is followed by a reversal of reaction. This increase appears also in peptone broth and is believed, as has been pointed out by others, to be due to a selective action upon that part of the peptone molecule which reacts like a carbohydrate.

The initial reaction of cultures of these organisms is one of the factors which exerts an influence in modifying the final reaction in any given medium.

Each organism appears to require a definite concentration of carbohydrate in a given medium if it is to produce its characteristic final reaction. In the case of B. carotovorus, the optimum concentration lies between 0.1 and 0.3 per cent with dextrose, saccharose, or lactose, and concentrations greater than 0.3 per cent are without effect upon final hydrogen ion concentration. The corresponding optimum of dextrose and saccharose for Bacterium tabacum, B. angulatum, B. glycineum, and B. sojae lies between 1.0 and 2.0 per cent. A concentration of glycerine between 1 and 2 per cent appears to be optimum for B. carotovorus. This optimum is determined by the reversal of reaction, such as occurs when insufficient fermentable sugar is present.

The minimum concentration of carbohydrate is related to the concentration of buffer materials since increases of peptone, through its increased neutralizing power, delays the production of the final level of acidity.

Fermentable sugars are not attacked with equal vigor, since the rate and amount of fermentation vary both with the species of organism and the kind of carbohydrate. B. carotovorus will, within the first 24-hour period, have developed a pH concentration from the fermentation of dextrose as great as that by any of the other species within 3 to 5 days. All, however, appear to be slow acting in contrast with animal pathogenes. B. glycineum ferments both dextrose and saccharose with considerable vigor, but not lactose and glycerine. With the latter two, there is a reversal of reaction even in a concentration of 2 per cent. B. campestre does not attack the sugars as evidenced by no increase in pH concentration in any of the cultures.

Mechanical agitation is decidedly beneficial to the growth of B. glycineum, probably due to aeration, the removal of by-products and renewal of food substances, as has previously been pointed out. With agitated cultures in 1 per cent dextrose the characteristic final pH concentration is attained, whereas with undisturbed cultures, there is a reversal of reaction.

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VI. The Application of Certain Recent Studies on Technic to Methods of Culture of Plant Pathogenes

Although certain general methods are employed in bacteriology, yet a specialized technic and special methods of experimentation have been developed in each of the several fields of this science variously designated as soil bacteriology, dairy bacteriology, hygiene and public health, sanitary engineering, medico-bacteriology, phytopathological bacteriology, etc. Furthermore, the methods employed in any one of these closely affiliated fields have been to a greater or less degree adapted to meet the needs of investigations in any of the others. As regards methods phytopathological bacteriology, it is believed, can with fairness be said to have borrowed from other fields of bacteriology more than it has contributed to them. If this assertion be true, it is due largely to the fact that this field of study is new and that investigators are not sufficiently familiar with the principles of general chemistry and biochemistry, and so are compelled to pursue cultural studies empirically.

Again, the general bacteriological methods for the study of the biochemical activities of bacteria which have been prepared by a committee of the Society of American Bacteriologists have come to be more or less standardized and unified, but, as has been necessitated by fundamental researches, have been modified from time to time. The present brief consideration is intended only to point out some recent improvements and refinements in technic which, it is believed, are applicable in cultural studies of bacterial plant parasites, and it is not intended in any way to interfere with the work or prerogatives of the Committee on the Descriptive Chart, nor in any sense to make suggestions or recommendations to them. No attempt will be made to enter into a comprehensive discussion of any of the points, many of which are covered quite fully, either in the several foregoing papers or in the work of others to which reference has been made in connection with these papers.

REACTION

Measurement of acidity. It is to physical chemists that credit is due for the development of methods of precision for the measurement of acidity. So many reasons have been urged against the employment of titrable acidity as determined by Fuller's scale instead of true acidity or hydrogen ion concentration as may be determined by the colorimetric method with a degree of accuracy sufficient for bacteriological work that it appears useless to devote further space to this subject. If as Clark (1) points out, 1 cc. of N 100 HCl is added to one liter of pure water of pH=7.0 the acidity of the solution becomes about pH=5.0. If, however, the same amount of acid is added to beef infusion broth of the same initial reaction, the resultant change is hardly appreciable. Equally striking is the observation of Meacham and his associates (2) on unbuffed media like corn meal extract in which 2 cc. of normal alkali in 1 liter changed the pH from about 4.0 to 10.0. In view of these observations, of many others of a similar nature, and of the fact that the Committee of the Descriptive Chart (3) has recommended the colorimetric method as more logical, of more significance, and as simpler, phytopathologists will, in accord with other investigators in bacteriology, find no objection to the abandonment of the titration method.

Adjustment of Reaction.—In the routine preparation of media according to usual methods, the medium is rendered neutral after the materials have been dissolved, is then boiled, after which it is adjusted to the desired pH concentration and is finally sterilized. In objection to this procedure, as Clark (4) has shown, it makes considerable difference whether alkali is added to bring the medium to the neutral point, and it is then adjusted to the desired acidity from this point or whether adjustment is made by introducing only a fraction of the alkali. Then, too, if adjustment is made as in the present studies only after the media have been cooled, flocculation, such as occurs in meat infusion and peptone broths with consequent change in buffer content, is prevented. Strongly acid or alkaline solutions and the employment of aseptic precautions will best serve for the adjustment of reactions. If finally media are not reheated after adjustment, changes in hydrogen ion concentration are avoided.

FERMENTATION

Preparation of carbohydrates. It is easily conceivable that a sufficient amount of reducing sugar could be formed from di- and polysaccharides when sterilized in a complex solvent like bouillon, so that an organism known to form acid from reducing sugars, but not from di- and polysaccharides could, in fermentation tests with these carbohydrates, produce an increase in acidity and thus lead to erroneous conclusions. In order to avoid this hydrolysis of carbohydrates, especially as occurs during sterilization in such complex solvents as culture media with its weakly acidic or basic properties, various investigations have employed concentrated carbohydrate solutions, prepared separately. They may be dissolved in distilled water, autoclaved at 10 pounds for 10 minutes and added aseptically to *cooled* sterile media in flasks. If then, the media are tubed or plated in sterile containers, the process of preparation of media containing sugars is both simplified and is in conformity with the best practice as determined by the laws of physical chemistry.

Concentration of carbohydrates. An optimum concentration of sugar, which can only be determined by experimentation, appears to be one of the factors involved in fermentation studies. One per cent of carbohydrate, the amount recommended by the Committee, (5) is manifestly insufficient for many plant pathogenes to permit acidification to proceed until the characteristic final hydrogen ion concentration is attained. If tests for changes in reaction are made at frequent intervals, a procedure which is very desirable, the production of small amounts of acids can then be detected and a reversal of the reaction, such as may occur in the presence of insufficient fermentable sugar, will not be the cause of error. The results of fermentation tests would, it is believed, be very much enhanced in value if data on duration of fermentation and amount of change in reaction were included. In connection with studies on concentration of carbohydrates, it has been noted that a governing influence is exerted by concentration of buffer and by initial reaction. In short, then, such data should be accompanied by a statement of the exact composition of the experimental media.

Kind of carbohydrates. Medical bacteriologists long ago realized that fermentation studies which involved only the four carbon compounds of the Descriptive Chart are very incomplete. They have, accordingly, made use of the "rare sugars" as shown in the routine separation of such closely related forms as members of the colon-typhoid-dysentery group. As an increasing number of plant pathogenic forms on the same or closely related hosts come to be known, as may be illustrated by the present studies with organisms associated with tobacco leafspot diseases, more emphasis will of necessity be placed upon the use of rare sugars. Their value for diagnostic purposes, even at the present stage of phytopathological development, appears to necessitate their employment.

Agitation. The oxygen relation is customarily studied in fermentation tubes. With certain organisms, clouding has been observed in the closed arm in tests, which when repeated, resulted in growth only in the open arm. The slight agitation resultant on handling has been noted to give an apparent growth in the closed arm. In consequence, inoculated fermentation tubes are left undisturbed for from 7 to 10 days, the period of duration of such tests.

If cultures are continuously agitated during fermentation, a marked increase in rate of fermentation and under certain conditions in amount also is produced.

THERMAL DEATH POINT

This is normally studied according to an accepted procedure. It has been shown though that variations in thermal death point may arise dependent upon hydrogen ion concentration. Furthermore, it is highly probable that. just as in studies on tolerance to acids, the kind of acid and character of the medium are factors which affect thermal death points.

NITRATE REDUCTION

Studies by Conn and Breed (6) have pointed out that several sources of error are inherent in nitrate reduction tests with standard nitrate broth. The poor growth of the colon organisms, the inability of Psuedomonas fluorescens to reduce nitrate in the presence of peptone, and the utilization of nitrate by B. caudatus as fast as it is formed, might lead to the erroneous conclusion that these organisms are unable to reduce nitrate. In view of the fact that these same causes may be operative in the case of many plant pathogenes which have been called non-nitrate reducers, exhaustive tests alone can determine ability to reduce nitrates.

TOLERANCE TO ACID OR ALKALI

The procedure usually employed in this phase of cultural studies of plant pathogenes yields little of value since so many factors to which no consideration has been given, are involved. Brightman, Meacham and Acree (7) have summarized this fact by stating that when measuring the limits of tolerance for hydrogen or other ions or molecules by organisms, care should be taken to state the concentration of chemicals and buffers used. When, therefore, data are accompanied by a statement of the exact composition of the experimental media which should include kind and concentration of acid or alkali, of buffers and of chemicals, they are reproducible and have a definite and distinctive value. Whether or not, however, such data can be correlated with problems of immunity and resistance to disease remains to be determined.

PREPARATION OF AGAR

Since the agar content of a medium through its effect upon viscosity profoundly influences the character of the colonies, a description of colonies as a means of specific identification is of value only when the concentration of agar is known. This factor has been largely overlooked since too often media are designated as plain agar, bean agar, corn-meal agar, dextrose agar, etc., and are unaccompanied by any statement of their percentage composition.

In the routine preparation of agar media, if the reaction is adjusted when they have been cooled to 50° to 60°C. and if vegetable or fruit decoctions are added at this temperature and they are not subsequently heated, there is no loss in jellifying power. The failure of such media as prune agar or rhubarb agar to solidify, an experience which many phytopathologists have encountered, may by this procedure, be averted. Solid media may thus be rendered very acid and can be employed in studies in which only liquid media have heretofore been available.

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THE GLOOMY SCALE

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**Conducted Jointly by the State Department
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RALEIGH

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THE GLOOMY SCALE

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INTRODUCTION

When the author first came to North Carolina, in 1908, he was impressed with the number of shade maples in a dead and dying condition along the streets, in lawns, in cemeteries—in fact, everywhere that maples had been planted for shade purposes. Even a cursory examination showed that this injury was confined to the soft maples, whereas the hard maples were practically free from the trouble.

Since 1908 the author has studied various phases of this problem in all sections of the State. The present bulletin is a summary of the more important phases of these problems and is published at this time to make the results of these studies available for other entomologists, and for landscape gardeners, keepers of parks, home owners—in fact, for every one who is interested in better shade trees, or in the preservation of shade trees already planted. The life history of the pest is dealt with at some length because this is the foundation for all methods of control. The remedies that can be used are discussed in detail, as well as the enemies of this scale, because it is believed that much benefit will be derived from both these sources.

IMPORTANCE OF THE GLOOMY SCALE

The gloomy scale (Fig. 1) is without exception the most important insect pest of shade trees in North Carolina. This is due not only to its destructiveness, but also to the fact that its chief host plants are so largely used for shade purposes in this State. The importance of this insect is, however, not always appreciated, chiefly because its attacks are so insidious, and because the effects of the attacks of this insect are slow to develop. It is usually several years after a tree has been attacked before the attention of the owner is aroused. By this time the attack has usually progressed to such a point that it is beyond control.

The importance of this pest can only be appreciated by one who gives close attention to the shade trees of the State, and then by watching the progress of the insect over a series of years. The writer of this bulletin has been watching the gloomy scale for the past thirteen years, and has been noting carefully the attacks of this scale on numerous trees throughout that period, and all these observations have convinced him that it is useless to try to grow soft maples in the gloomy scale territory, unless the trees are sprayed consistently at least every second or third year. A visit to almost any of our cities or towns will show that fully three-fourths of the shade trees are soft maples. Perhaps the reason for this is the fact that these maples grow rapidly and stand much abuse in

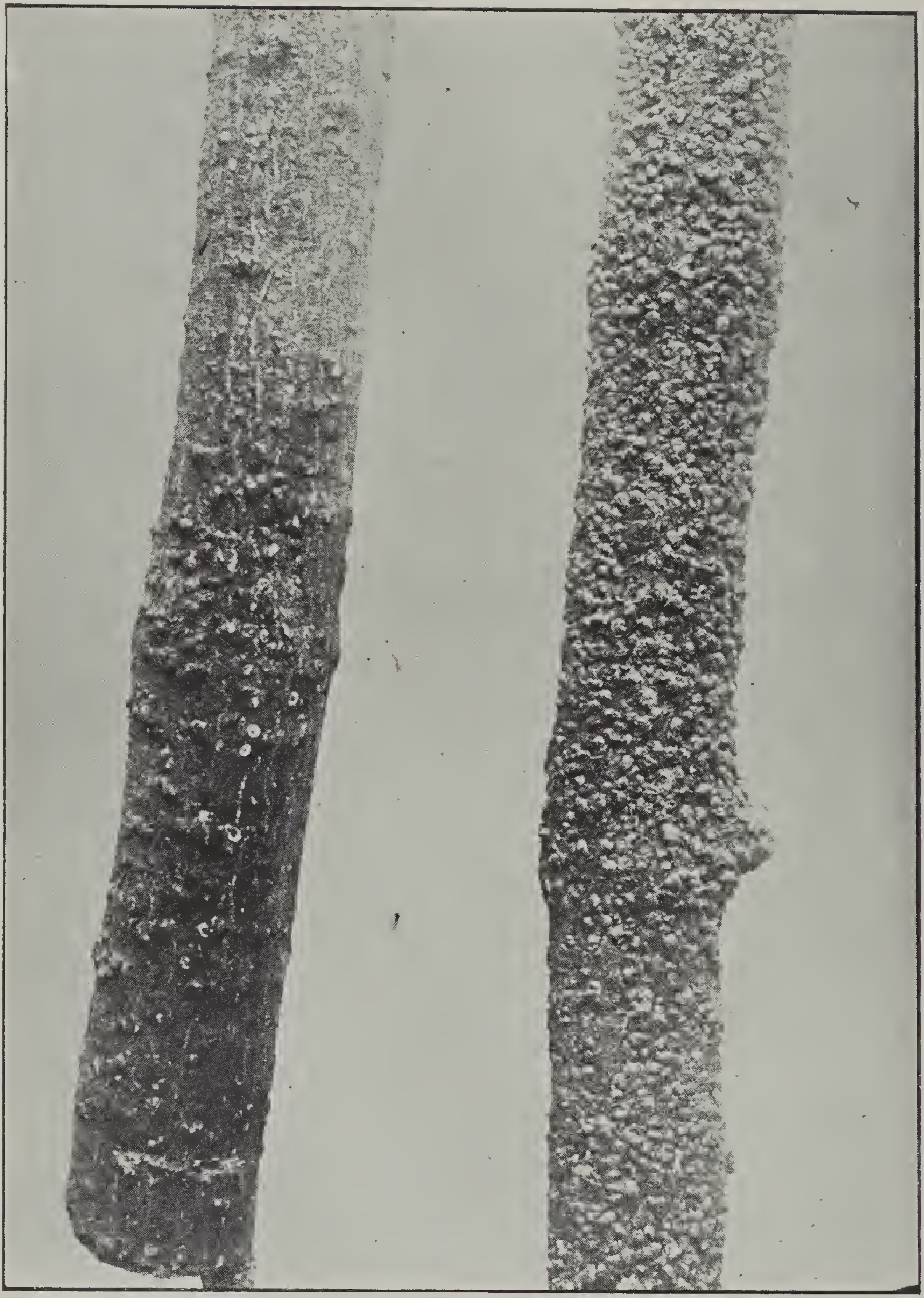


FIG. 1. Maple twigs infested with gloomy scale; slightly infested on the left, badly infested on the right. About twice natural size.

the way of mechanical injuries to trunk, limbs and roots. In spite of these advantages the soft maples have certain disadvantages in addition to the fact that they are attacked by the gloomy scale. The chief one is that the wood is especially brittle and apt to be broken during storms.

COMMON NAMES

So far as the writer is aware, only two common names have ever been used for this species. Comstock (1881), in his original description, proposed the name *Gloomy Scale,* and this name has been rather generally used ever since. The name *Maple Scale* has been used to a certain extent, but since that name does not distinguish this scale from other scales on maple, it is hardly advisable to use it. The name gloomy scale has also been used for another species, *Chrysomphalus obscurus* Comst., which occurs more commonly on oaks. This latter species was named obscure scale by Comstock, and this name should be retained, or the two species should be called the gloomy maple scale and the gloomy oak scale. The latter alternative is hardly advisable, for *Chrysomphalus tenebricosus* frequently occurs on oaks as well as maples, while *Chrysomphalus obscurus* often occurs on maples as well as oaks. The name gloomy scale is appropriate because the scale covering of the insect is very dark in color. With these things in mind, and the further fact that the name gloomy scale was originally proposed for *Chrysomphalus tenebricosus,* the name gloomy scale is retained for this species in this bulletin.

HISTORY AND SYNONOMY

This species was originally described by Comstock (1881) in the report of the United States Commissioner of Agriculture for 1880, as *Aspidiotus tenebricosus,* in 1897 Cockerell (1897) suggested that this species would fall in *Ashmead's* genus *Chrysomphalus* treated by Cockerell as a sub-genus of *Aspidiotus.* American writers have generally referred it to this genus, but Leonardi (1889) placed this species in his genus *Aonidiella.* Herrick (1911) has recently suggested that this species should be placed in the genus *Aonidiella* until the matter has been definitely settled by entomologists. The writer follows the usual custom among American entomologists and places this species in *Chrysomphalus Ashmead.*

The synonomy of the species would, therefore, be as follows:

Aspidiotus tenebricosus, Comstock 1881.
Aspidiotus (Chrysomphalus) tenebricosus, Cockerell 1897.
Aonidiella tenebricosus, Leonardi 1899.
Chrysomphalus tenebricosus, Fernald 1903.
Chrysomphalus (Aonidiella) tenebricosus, Herrick 1911.

The original description by Professor Comstock (1881) is exceedingly careful in detail as far as the structure of the insect goes, but at that time nothing was known of the life history or the habits of the insect. Professor Comstock's description and notes were repeated by Packard in 1890. Craw, in 1891, reported the insect as occurring in California, and Lintner, in 1896, mentions the insect very briefly. In 1911 the writer discussed the insect briefly and gave a detailed account of test sprayings that had been carried on against it. Herrick, in 1911, also gave the technical description of the insect, with the statement that it is "abundant nearly everywhere on the silver and red maples, which were often dying as a result of the severe infestation." Further than this the insect has been little noted in entomological writings, although entomological workers in the southern states generally appreciate its destructiveness.

DISTRIBUTION

The gloomy scale is generally distributed throughout the southeastern United States, from Maryland to Texas. From records and observations of entomologists from other states it is apparent that this insect does not occur normally north of Delaware, Maryland, Virginia, Tennessee, and Missouri. Its western distribution is not clear. It has been recorded by Craw from California, but has apparently not been found in that state in any recent collections. There are a few scattered records of this insect in states farther north than the boundaries indicated, but apparently these records are based on accidental or unusual occurrences. Scale insects have been collected extensively in Ohio, Indiana, Kentucky, and Kansas without revealing the presence of this

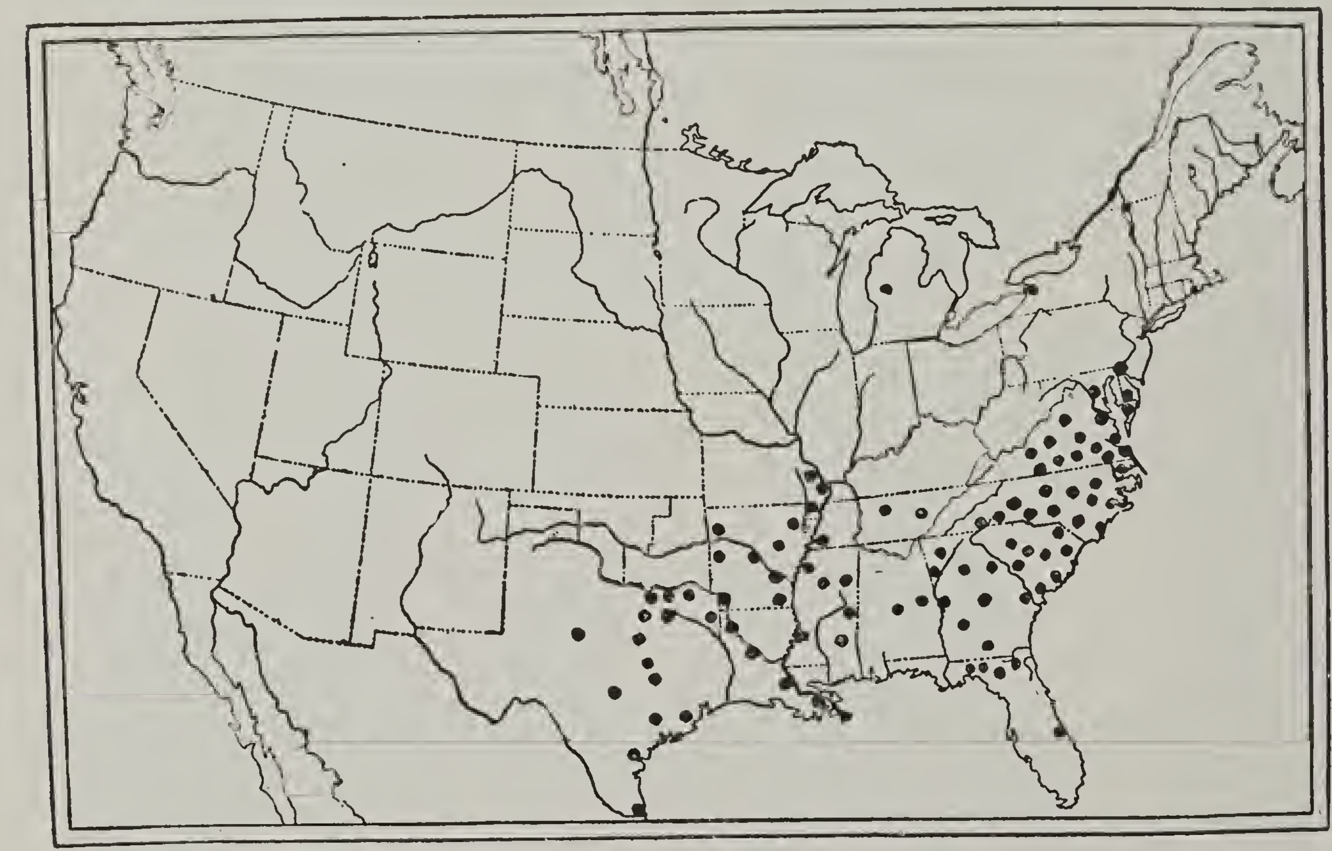


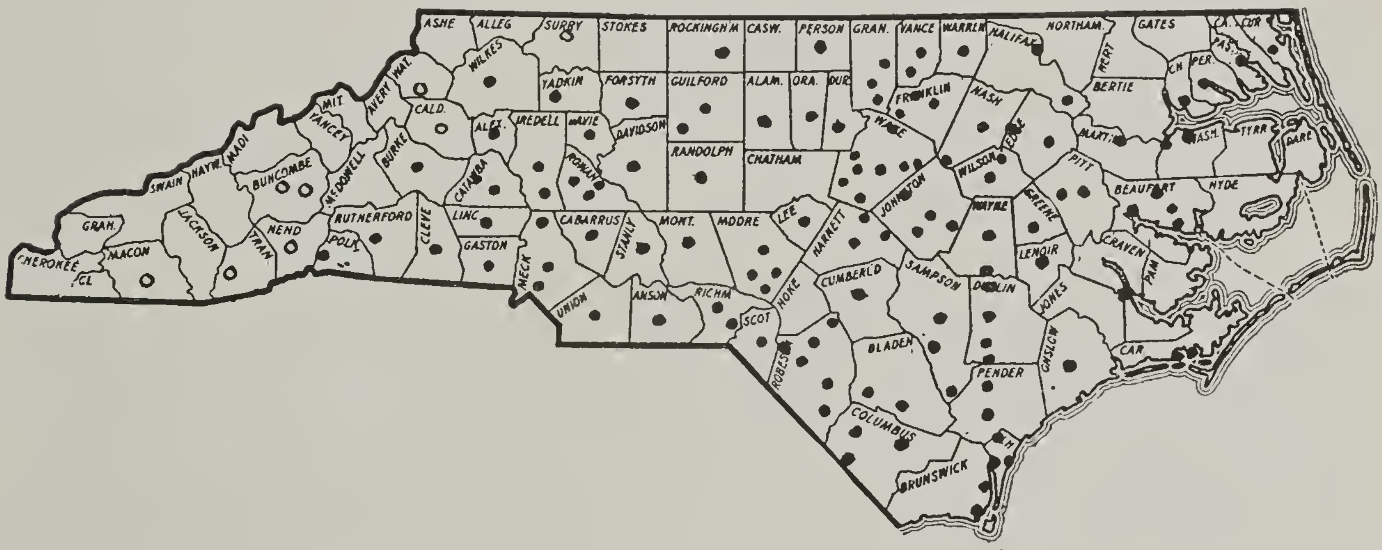
Fig. 2. Map of the United States showing the recorded distribution of the gloomy scale.

insect. In the states south of this line, however, the insects seem to be generally distributed in cities and towns wherever maples have been used extensively for shade purposes. The map given in Fig. 2 shows very clearly the distribution of this insect.

For the records outside of North Carolina my thanks are due to Mr. E. R. Sasscer, who has kindly sent me the records from the United States Bureau of Entomology, and to the entomologists of various states in the gloomy scale region who have very kindly summarized the conditions in their states.

DISTRIBUTION IN NORTH CAROLINA

In North Carolina the gloomy scale does not occur beyond what is generally accepted as the western boundary of the Upper Piedmont. The map in Fig. 3 indicates very well the distribution of this insect in the State. The records are based upon correspondence and careful inspection of all of the large cities and towns of the State. Its prevalence in the eastern part of the State is apparently dependent upon the extent to which soft maples have been used for shade purposes. In all of the larger cities and towns in the eastern part of the State it is practically impossible to find soft maples of any size which are not seriously infected with the gloomy scale.


ASHE
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GATES
CA.
CUR
PAS.
CH
PER.
BERTIE
MARTIN
WASH.
TYRR
DARE
BEAUFORT
HYDE
WAT.
AVERY
MIT
YANCEY
MADI
HAYW.
SWAIN
GRAH.
CHEROKEE
CL
MACON
JACKSON
TRAN
HEND
BUNCOMBE
McDOWELL
BURKE
CALD.
WILKES
ALEX.
YADKIN
FORSYTH
GUILFORD
ALAM.
ORA.
DUR
FRANKLIN
NASH
EDGE
WAKE
IREDELL
DAVIE
DAVIDSON
RANDOLPH
CHATHAM
ROWAN
CATAWBA
RUTHERFORD
POLK
CLEVE
LINC
GASTON
MECK
CABARRUS
STANLY
MONT.
MOORE
LEE
HARNETT
JOHNSTON
WILSON
WAYNE
GREENE
PITT
LENOIR
CRAVEN
PAM
JONES
UNION
ANSON
RICHM
SCOT
HOKE
CUMBERL'D
SAMPSON
DUPLIN
ONSLOW
CAR
ROBESON
BLADEN
PENDER
COLUMBUS
BRUNSWICK
N.H

FIG. 3. Map of North Carolina. The solid dots indicate places where the gloomy scale has been found. The small rings indicate places inspected, but no scale found.

An effort has been made to determine the western extent of this scale, and certain towns along the border have been visited with this in view. The localities in which it has not been found are also indicated in the accompanying map. We may look, therefore, for this scale everywhere in the Piedmont and Coastal Plain sections of the State where soft maples have been planted extensively.

NATIVE HABITAT

In the present day of world-wide commerce the native habitat of any species of insect becomes of interest. Due to the introduction from foreign countries of several very destructive pests, the opinion has become rather prevalent that all destructive pests are introduced from abroad. The writer, however, believes that the gloomy scale is a native species. His reasons for believing this are based on the following facts: (1) The gloomy scale is found frequently in woods far removed from towns or cities where infestation is general; (2) the gloomy scale has never been reported from any other country than the United States, so far as the writer is aware. While neither one of these facts is conclusive, they both point the same way. The finding of this pest on native maples growing wild, both in the foothills of the mountains and in the swamps in the eastern part of the State, frequently several miles from human habitation, would be hard to explain on any other basis. The further fact that scale insects have been rather generally studied in all parts of the world and the gloomy scale has not, so far as a careful search of the record shows, been reported from other countries, can lead to but the one conclusion—that this is a native pest.

HOST PLANTS

The gloomy scale attacks a long list of trees, as is shown by the following list, but it is chiefly an enemy of the soft maples, red maples (*Acer rubrum*), and silver maple (*Acer saccharinum*). The other trees in the list are usually attacked only when they are placed close to soft maples so that they could hardly escape. Hard maples, such as the sugar maple (*Acer saccharum*), and the Norway maple (*Acer platanoides*), are very resistant (Fig. 4), even when placed in direct contact with dead or dying soft maples.

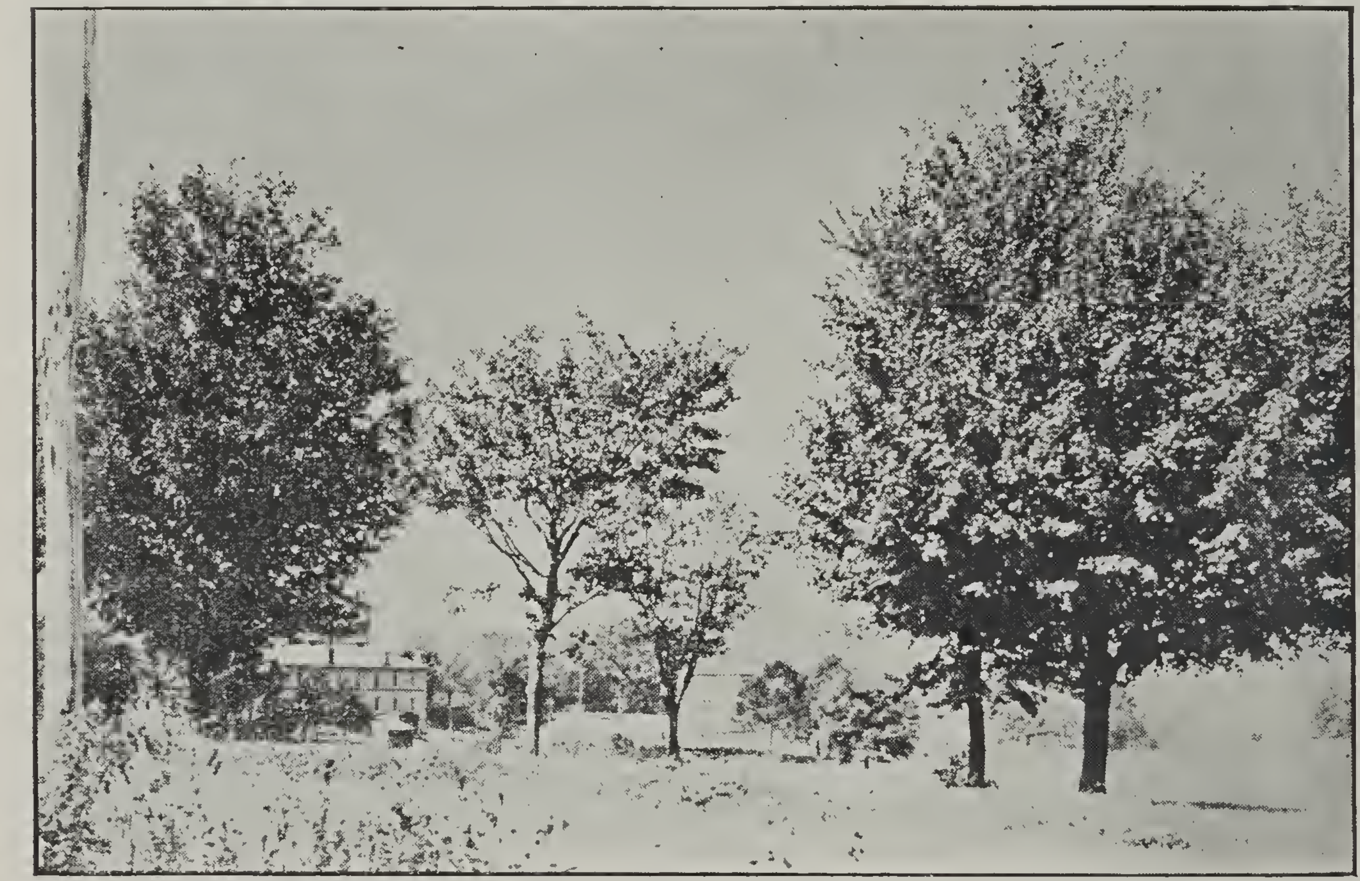

FIG. 4. Showing the resistance of the hard maples to the attacks of gloomy scale. The trees on either side are hard maples; the trees in the center are soft maples.

LIST OF HOST PLANTS

Apple (*Pyrus malus* L.). Occasionally infested.
Ash, white (*Fraxinus americana* L.). Bureau of Entomology.
Box-elder (*Acer negundo* L.). Occasionally slightly infested.
Buckeye (*Æsculus glabra* Willd.). Slightly infested.
Chestnut (*Castanea* spp.). Several badly infested trees.
Cottonwood (*Populus deltoidea* M.)
Currant (*Ribes* sp.). Bureau of Entomology.
Dogwood (*Cornus* spp.). Occasionally infested in the woods.
Elm (*Ulmus* spp.). A few slightly infested trees.
Gall-berry (*Ilex* sp.). J. R. Watson.
Hackberry (*Celtis* spp.). Slightly infested.
Hibiscus. Bureau of Entomology.
Iron-wood (*Carpinus caroliniana* Walt.). A single badly infested tree.
Linden (*Tilia americana* L.). Bureau of Entomology.
Maple, Norway (*Acer platanoides*). A few slightly infested trees.
Maple, red (*Acer rubrum* L.). Generally and badly infested.
Maple, silver (*Acer saccharinum* L.). Generally and badly infested.
Maple, sugar (*Acer saccharum* Marsh.). A few scattering individuals found infested very slightly.
Mulberry (*Morus* spp.) Sometimes very badly infested.
Oak, live (*Quercus virginiana* L.). Bureau of Entomology.
Oak, water (*Quercus aquatica* Catesb.). Sometimes slightly infested.
Oak, willow (*Quercus phellos* L.). Sometimes slightly infested.
Oak, white (*Quercus alba* L.). Sometimes slightly infested.
Osage Orange (*Maclura pomifera* Raffin.). Sometimes badly infested.
Sarsa sp. Bureau of Entomology.
Sycamore (*Platanus occidentalis* L.). Sometimes slightly infested.
Tulip (*Leriodendron tuliplifera* L.). Sometimes slightly infested.
Walnut (*Juglans* spp.). Some trees badly infested.
Willow (*Salix* sp.). Sometimes slightly infested.

APPEARANCE OF THE INSECT

Armored scales differ from other insects with which most of us are familiar because they live between two scales or coverings like a turtle in its shell. They differ from a turtle in that the insect is not attached to the scales, whereas in the turtle the animal is attached to its shell. They differ from other insects also in that they do not have legs or wings in their feeding stages; hence they cannot move from place to place, but are anchored on the bark of their host plant by means of their thread-like beaks.

If we examine closely a twig of a maple tree which is lightly infested with gloomy scales we will find small circular elevations scattered about, which have almost the same color as the bark (Fig. 1). If we examine a badly infested twig we will find these little elevations closely crowded together so that they completely cover the surface of the bark (Fig. 1).

Now, if we take a pin or the small blade of a knife, we will find that these small elevations can be lifted up and under some of them we will find a small yellow sack-like structure, the insect itself. If we touch the insect carefully we see that it is anchored to the bark by its beak. Under the insect we will find a circular whitish area of wax, the ventral scale.

LIFE-HISTORY SUMMARY

The gloomy scale, in common with all the other armored scales, has two entirely distinct life histories, one for the female and the other for the male. Individuals of both sexes start their development as minute six-legged insects, with a pair of eyes and a pair of antennæ. These are called active young, and do not differ in the two sexes; but from this point the development in the two sexes is widely different.

In the female the active young soon settles, inserts her beak into the bark and commences to suck the sap of the host tree. Slender thread-like waxy filament exude from the wax pores located over the body, and as these mat together, two plates of wax are formed, one covering the back of the tiny insect and the other between it and the bark of the tree on which it is feeding. The insect is soon covered in this way, and in a short time it molts, loosing its eyes, legs, and antennæ. Thus the female becomes a sack-like animal with its beak inserted into the bark for sucking out the sap. It is not capable of locomotion, and from this stage onward the female is forced to remain in the same place. This development is taking place during the summer months. In the late summer the females are fertilized by males. They molt again and pass the winter as half-grown individuals. In the spring they grow rapidly for a short period and early in May they commence to give birth to living young, which start another cycle. This process is kept up during the whole summer. The mature females diminish gradually during the summer, the last ones dying in late August.

The males start from active young like the females and form their scale coverings and molt in the same way, but after the first molt they are more elongate than the females and the scale covering is oval in outline, not circular as in the female. The male at this stage is an elongate sack-like insect, feeding on the sap through the long, slender beak. About the middle of August the males change remarkably. They loose their mouth parts and cease feeding. Three pairs of legs, a pair of antennæ, and a pair of wings develop as sack-like outgrowths of the body. At first these appendages are poorly formed, and this stage is

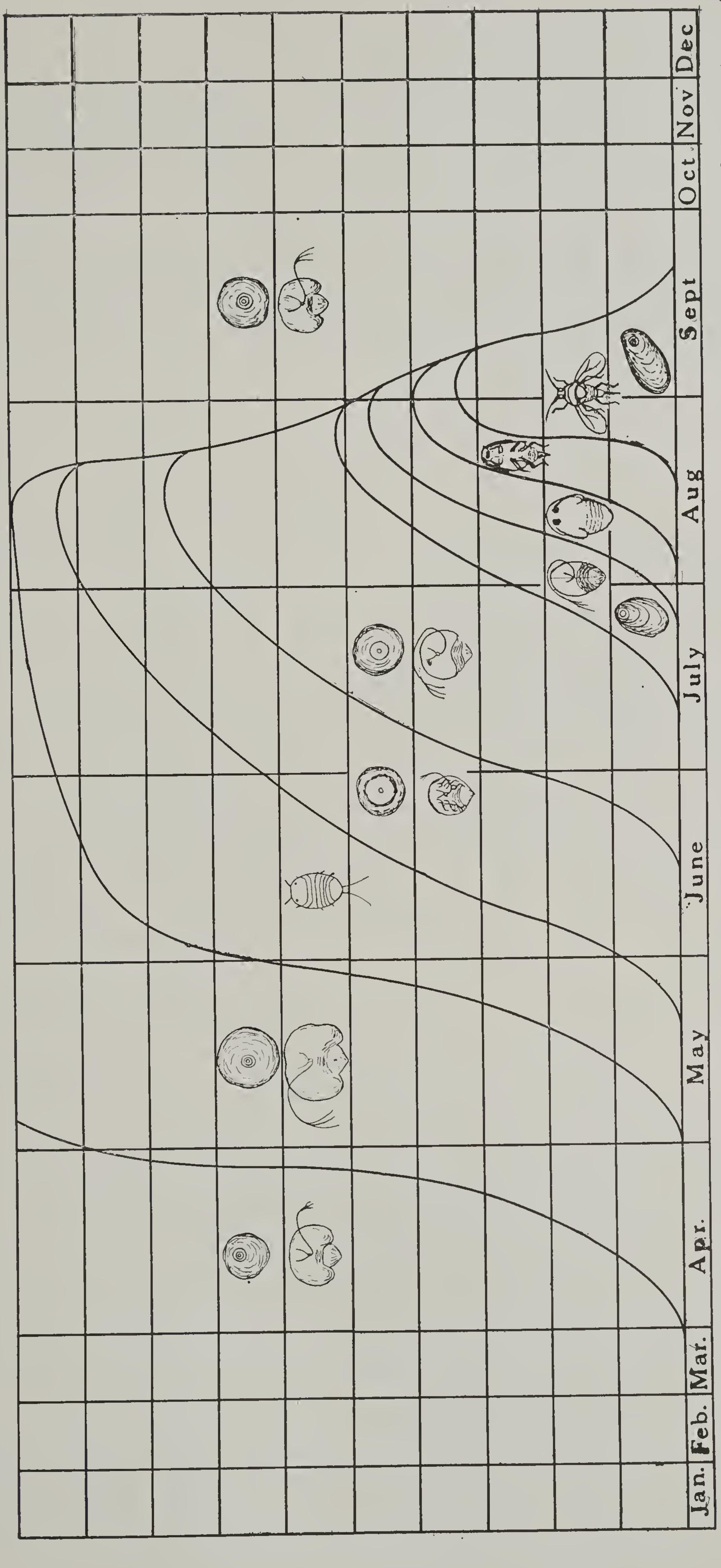

FIG. 5. Life-history summary based on counts of the living insects at various seasons of the year. The vertical lines represent the percentage of any stage found at any given season.

known as the pro-pupa, but later they are very definitely outlined, and the minute insect resembles the adult very closely. This stage is known as the pupa. The pupa soon changes to an adult with legs, antennæ, wings, and eyes fully developed. It emerges from under the scale covering and flies away to mate with the developing females, after which it dies.

ACTIVE YOUNG

Description. The active young gloomy scale (Fig. 6) is a minute six-legged insect, provided with a pair of antennæ and a pair of eyes. The general color is pale straw-yellow, with the eyes dark brown.

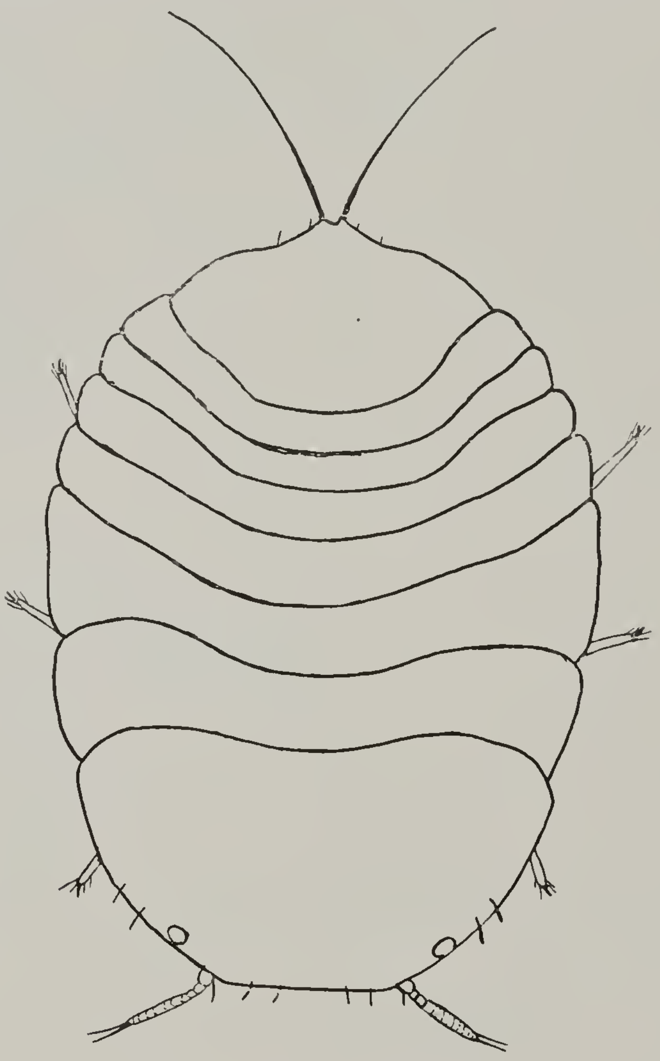

FIG. 6. Active young.

Length .244 mm., width .166. The general shape of the body is broadly oval, with legs, antennæ, and posterior spines showing conspicuously. The body is plainly segmented, showing seven evident segments. The head bears the antennæ, which are four-jointed. The first joint is about twice as long as the second and third together. The second and third are sub-equal. The fourth is about three times as long as the first, second, and third together, and is indistinctly annulate. There is a strong spine on the mesial border of the basal segment and two long spines on the apex of the fourth joint.

Habits. The active young are minute six-legged insects of a pale straw color. They are provided with a pair of dark-colored eyes and a pair of antennæ. The active young of the gloomy scale are born alive, but in a rather inactive condition. They usually remain under the scale of the female for from 24 to 48 hours, although some remain much longer than this. Usually during the height of the breeding season, anywhere from one to ten active young may be found under each female. These usually fare forth after a time, crawling about on the limbs and branches of the trees until a convenient location is found. When such a location is found they attach themselves by inserting their beaks and sucking the sap. This active period is usually of short duration, averaging about one hour in the specimens studied. As a general proposition these active young do not travel far, although if conditions are unfavorable they may travel a considerable distance. Usually the young scales settle within a foot of their mother. This leads to great crowding of the scale, and results in the death of many, due to the red-headed fungus. Those that leave their mother for any distance travel towards the tips of the branches, where they settle for the most part in the axils of the leaf in the new growth. This insect would be much more destructive than it is if all the young adopted this method of attacking the young growth, for they would not be so crowded, hence fewer would die; and what is perhaps of greater importance, the young tip twigs would be killed.

The greatest measured distance that young scales have traveled for us is 56 inches, measured as a straight line. This young scale was removed from under its mother and placed on a young maple tree out of doors. The scale was liberated near the base of the tree and crawled upward and outward on a limb, near the top of the tree. No account was taken of the innumerable turnings and twistings of this young, but the distance measured as a straight line was about 56 inches. It is safe to say that the real distance traveled was about 100 inches (2500 mm.) in three hours, or an average of more than 8,000 times its own length in an hour. Usually, however, the young find a satisfactory location and settle within a few inches of their mothers. It is not unusual to find an adult female, with a ring of young scale around her. Frequently the active scales crawl under old male scales or dead female scales and settle there. It is not unusual to find as high as three generations overlapping each other in this way. It is of interest to note that these scales underneath other scales are grayish white in color, showing that light is required to develop the characteristic color of the adult scale. It is not unusual for young scale to settle on the dorsal scales of other individuals. And while their mouth parts cannot reach the bark in this position, they frequently secrete a dorsal and ventral scale covering and molt the first time, after which they die. This shows that the young carry enough food and energy to develop to a remarkable extent, although deprived of food. In the laboratory we have not been able to keep the young active for more than forty-eight hours without food. Apparently their energies are all used up in aimless endeavors to escape from their cages.

Soon after settling and inserting the beak, the active young contracts its antennæ and legs and the body contracts until it is almost circular in outline (Fig. 5). Filaments of wax are secreted from all parts of the body, the waxy filaments soon run together and gradually melt, so that the downy appearance that is so characteristic at first is soon lost. The color of the wax is white at first, but later it turns a very dark grayish black.

DESCRIPTION OF FEMALE STAGES

After the *first molt* (Fig. 5) the female scale insect is nearly circular in outline and measures about .375 mm. in diameter, and except for its small size it resembles the adult female very closely. After the second molt the female scale insect is about .510 mm. in diameter.

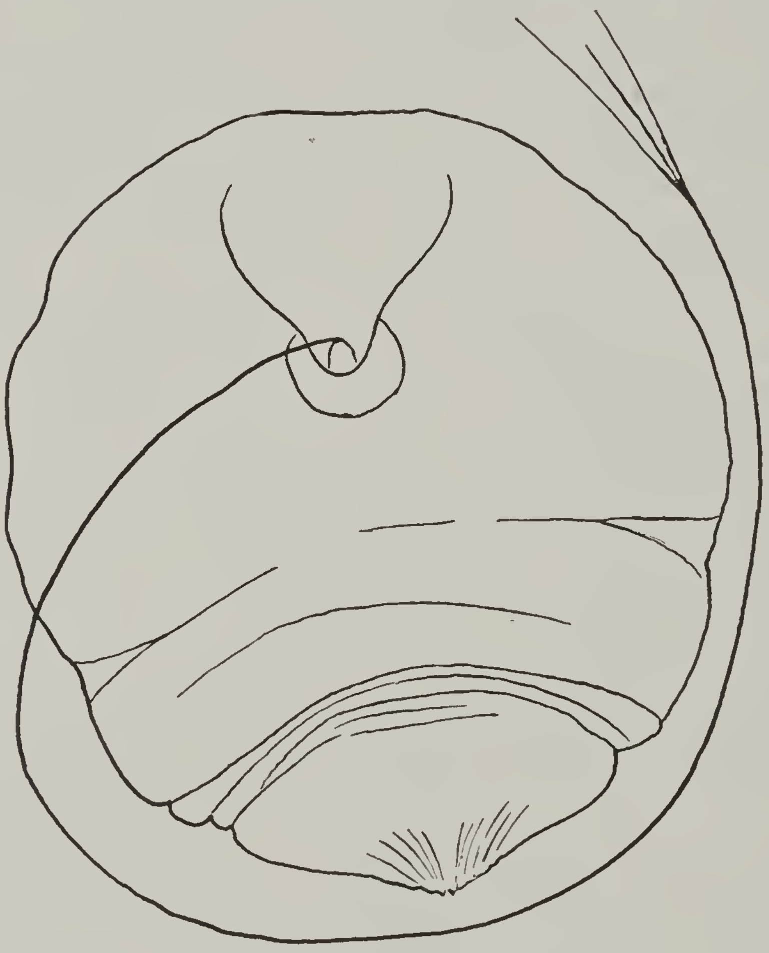

FIG. 7. Adult female insect.

The *adult female* (Fig. 7) measures about .950 mm. in diameter, but very slightly longer than broad. The mouth parts are nearly central in position. The segments of the abdomen are not very distinct. The general color as chitin yellow. The pygidium (Fig. 8) shows the following characters:

"There are three pairs of well-developed lobes. The median lobes are rounded posteriorly, or often with a slight notch on the lateral margin, and taper to a point anteriorly; the second lobe of each side is somewhat triangular in outline, with the lateral edge serrate; the third lobe is larger than either the first or second lobes, triangular in outline, and serrate on lateral margin.

"The posterior third of the lateral margin of the segment appears to be of the same structure as the lobes, and has five triangular serrate lobes; the posterior one of these is the largest, and is larger than either of the true lobes.

"There are seven club-shaped thickenings of the body wall upon each side of the meson, which are arranged as follows: One terminating near the lateral margin of the first lobe; this extends anteriorly but a short distance beyond the lobe. One appearing to be a prolongation of the mesal margin of the second lobe; this extends anteriorly to a point laterad with the anus. One terminating between the second and third lobes; this is linear, inconspicuous, and sometimes obsolete. One terminating at the base of the plates between the second and third lobes, and also one terminating at the base of the plates between the third lobe and the thickened lateral margin; these two are the largest, and extend anteriorly the farthest of all the thickenings. One terminating at the mesal margin of the third lobe, and one at the mesal end of the thickened lateral margin of the segment.

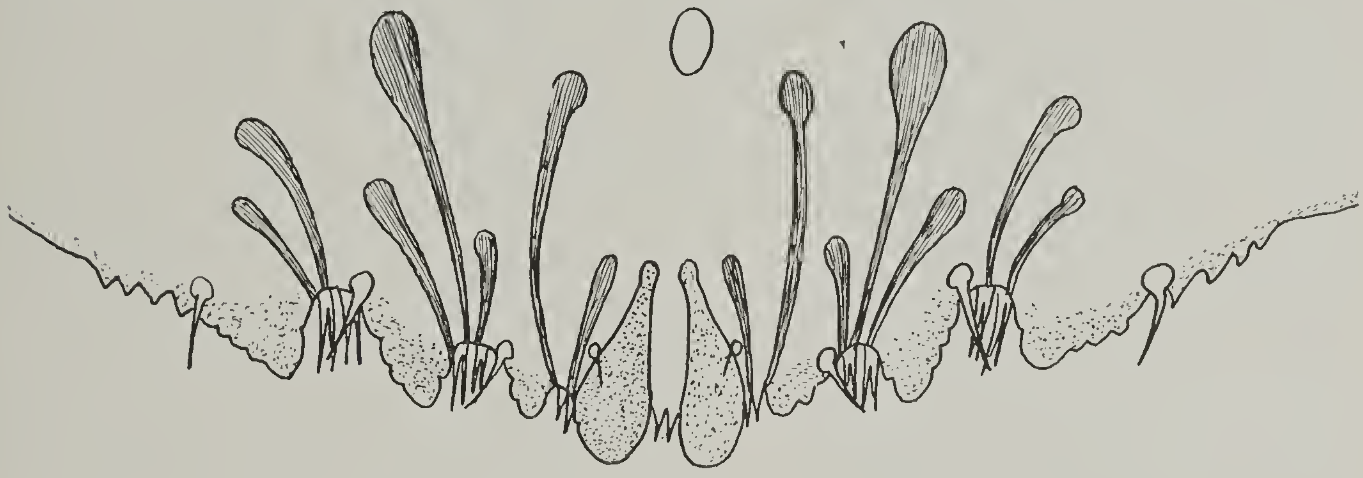

Fig. 8. Pygideal fringe of gloomy scale (*after Herrick*).

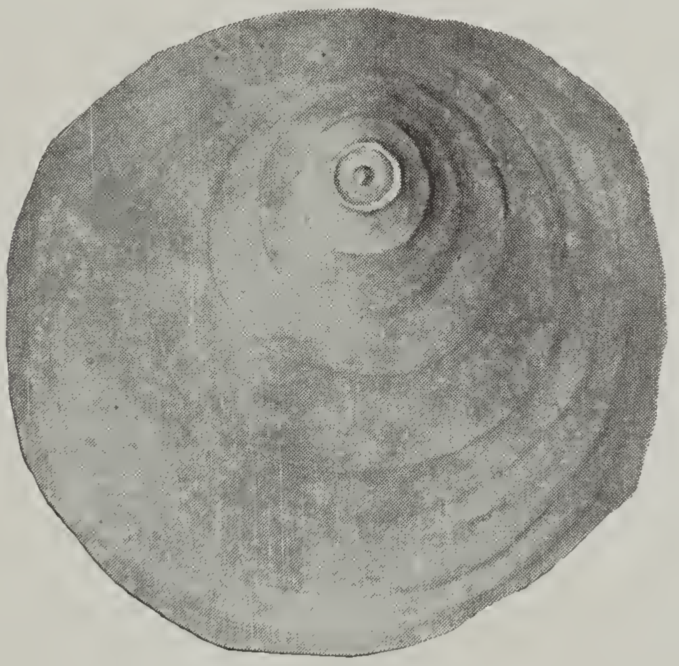

Fig. 9. Dorsal scale of female.

"The plates between the median lobes and between the first and second lobes of each side are very small and often obsolete; there are two small irregularly branched plates between the second spine and the third lobe, and also two similar plates between the third spine and the mesal end of the thickened lateral margin.

"There are five pairs of spines on the ventral surface of the segment. and six on the dorsal. Those at the base of the median lobes are very small; the others are conspicuous. The second and third spines of each surface are situated just laterad of the second and third lobes respectively; in each case the dorsal spine is slightly mesad of that on the ventral surface. The fourth spine of the ventral surface is on the penultimate lobe of the thickened lateral margin. The fifth spine of this surface is near the anterior end of the thickened part of that margin. The fourth and fifth spines of the dorsal surface are in each case mesad of the corresponding spines of the ventral surface. There is also a spine on the dorsal side, very near the penultimate segment.

"The scale of the female is very dark gray, agreeing in color with the bark to which it is attached; the protuberance indicating the position of the exuviæ is marked with a white dot and concentric ring (Fig. 9); in rubbed specimens this protuberance is smooth and black, in all cases the remainder of the surface of the scale is rough. The scale is very convex; the exuviæ are usually between the center and one side. The ventral scale is well developed, especially at the margin, where it is much thickened and is dark colored; the central part is white and adheres to the bark, while the thickened margin is easily removed as a ring. Diameter of scale, 1.5 mm. (.06 inch)." (Comstock.)

DESCRIPTION OF MALE STAGES

First stage. The scale covering is similar to the adult male scale covering and measures 1.0 mm. (1/25 in.) in length by .750 mm. (1/32 in.) in width. The insect is more elongate than that of the female (Fig. 5) and measures about .325 mm. in width and .451 mm. in length; otherwisewise it is similar to the female second stage.

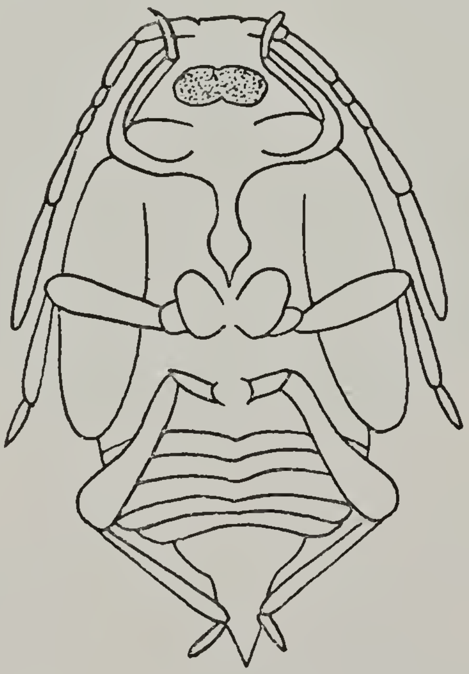

FIG. 10. Pupa of gloomy scale.

The *pro-pupa* (Fig. 5) measures about .577 mm. in length and about .420 mm. in width. The color is strong chitin yellow. The general shape of the body is broadly triangular, with the head narrow. The antennæ, legs, and wings are developed as sack-like outgrowths of the body.

The *pupa* is pale chitin yellow in color, with the legs, wing pads, antennæ, and style well developed (Fig. 10). The antennæ reach to almost the base of the abdomen. The forelegs are folded forward, with the tips of the tarsi reaching the base of the antennæ. The femora of the middle legs project laterad and the tarsi are bent toward the end of the abdomen. The hind femora project backwards, with the tarsi bent at almost right angles. The style is short, measuring about .10 mm. in length.

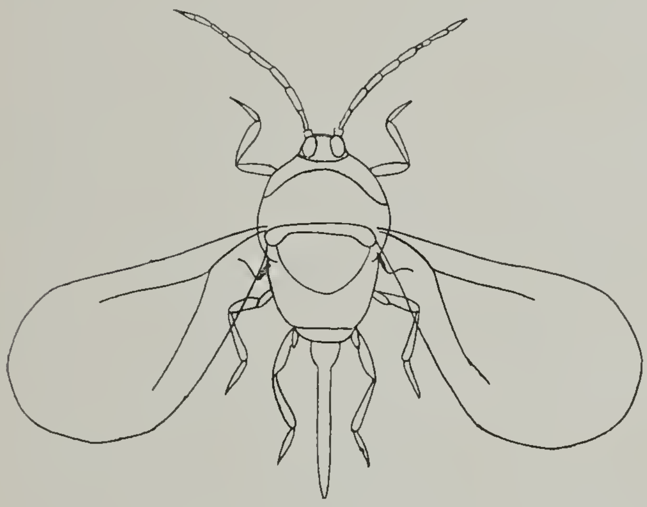

FIG. 11. Adult male gloomy scale.

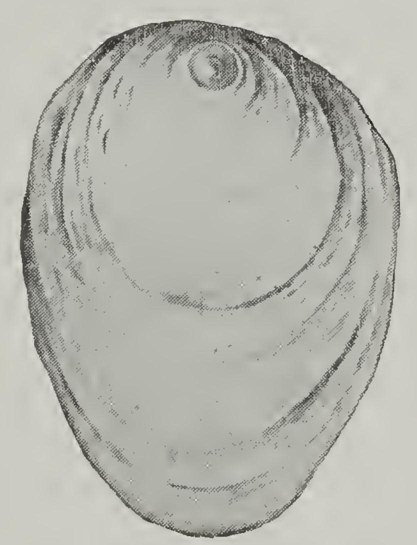

FIG. 12. Dorsal scale of male gloomy scale.

The *adult male* is a strong chitin yellow in color, with head and eyes darker (Fig. 11); the wing expanse is about 1.60 mm.; length of body, including the style, .75 mm.; style long and slender, about .24 mm. long; the antennæ are as long as the body, ten-jointed; basal joint short, scarcely half as long as second; second, third, and fourth sub-equal, which are one-half longer than fifth and sixth; seventh and eighth half as long as fifth; ninth as long as seventh and eighth together, broader; tenth about three-fourths as long as ninth, gradually tapering to a fine point. The antennæ are covered with short, sparse hairs. The head is small, with the lateral eyes situated just laterad to the antennæ; the diameter of the ventral eyes is about twice that of the lateral eyes. The prothorax is crescent shaped, about one-third as long on the middle as the mesothorax. The thoracic band is brownish in color and rather broad. The metathorax is about one and a half times as long as mesothorax and considerably inflated. The abdomen is flat and expanded.

The scale of the male (Fig. 12) is broadly oval in outline, of the same color as the female scale, and measures about .910 mm. in length and about .0680 mm. in breadth at the broadest part. The larval skin is near the anterior end. The ventral scale is white, with the margins darker and somewhat thicker.

HABITS OF THE MALE

After the male scale has reached the adult condition it remains under the scale covering for several days in the perfect state before it emerges. After the insect emerges from the scale it stretches its legs and expands its wings and generally deports itself like a person aroused from a sound sleep. Afterwards it walks about over the twigs, searching for suitable females. It taps the female scale covering with its antennæ and seems to be able to locate desirable mates in this manner. The males do not fly away from the place where they emerged and mate with distant females, as described by Quayle for the Red Scale, but they seem to mate with the first suitable female. In our cages the males have always died after mating only once. This can hardly be the rule in nature, however, for the proportions of females to males seem to be about two to one.

NATURE OF THE DAMAGE

The gloomy scale injures the tree by sucking the sap from the trunk, limbs, and branches. This sap carries the mineral foods and water from the soil through the roots, trunk, limbs and branches to the leaves, where it is manufactured into complex substances which are used by the tree in forming new growth, new leaves, seeds, etc. Now we can kill a tree by girdling it, that is, by cutting out a ring of bark all the way round the trunk. Thus we cut the flow of sap from roots to leaves. The gloomy scale has the same effect as girdling, by sucking the sap. A few insects will not hurt the tree appreciably, but if they become numerous enough they will kill small twigs or branches. If still more numerous, they may kill the tree outright.

The first signs of the injury of a tree by the gloomy scale is that small twigs die. These are perhaps most noticeable early in the spring when the leaves are putting out. As the attack progresses, larger limbs die, and the tree has the appearance shown in Fig. 13. That is, instead of the head being thick and dense, it is very thin. About this same time the tree commences to throw out suckers around the base. Trees apparently linger in this condition for years if other conditions are favorable, but in this stage they readily succumb to slight mechanical injuries to roots, trunk, or larger branches. In other words, the tree may have vitality enough to exist, but not enough to stand any additional shock.

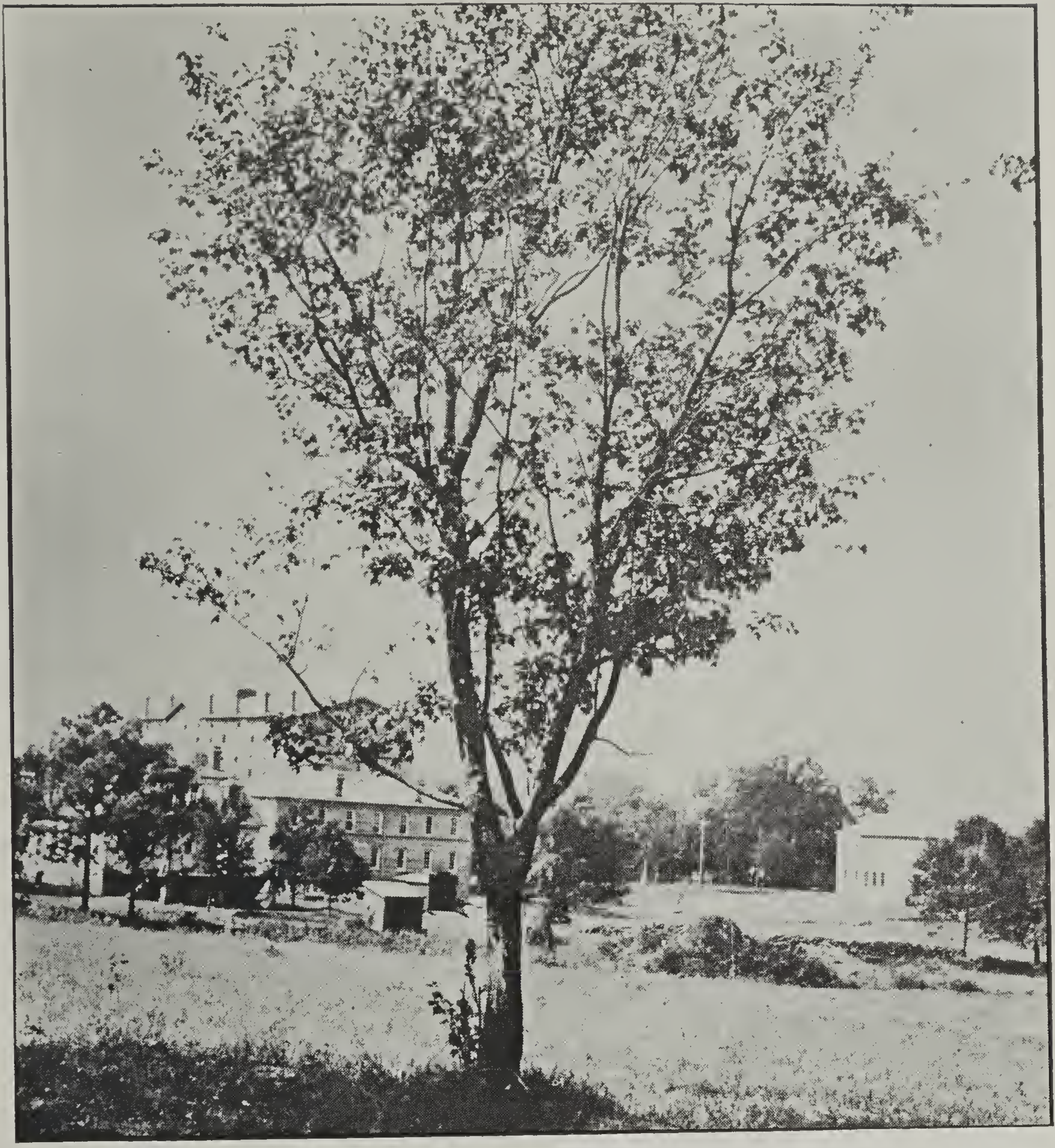

FIG. 13. Soft maple badly infested with gloomy scale.

HOW THE INSECT IS SPREAD

There are two factors in the spread of this insect: the spread from place to place, and the spread from tree to tree. The gloomy scale is so generally distributed in eastern North Carolina that the spread from place to place is not of so very much importance. However, it seems to take place chiefly through the nurseries. Nurserymen try to keep this pest in control, but it is of course a rather difficult matter to control them absolutely. And a single live insect would soon increase to such an extent that the pest would seriously injure the tree.

The spread from tree to tree may take place in a number of different ways. The active young may crawl from the limb of one tree to the limb of another if the limbs interlace. They may be blown from one tree to another, as they are very small and light. The young scale may also be carried from one tree to another on the legs and feet of birds, although this method must be rather rare. On the whole, the insect must rely principally upon favorable winds to spread it from tree to tree.

ENEMIES

The gloomy scale is subjected to the attacks of a number of enemies, the chief one being a small parasitic wasp which attacks the young females during midsummer especially. This insect is perhaps the chief check on this pest, although there are a number of other parasites that prey upon it. It is very difficult to appreciate the good that these parasites do, because they are so small and their work is hidden under the scale covering. The work of these minute insects may be detected by the neat circular holes which they cut through the dorsal scale covering in making their escape.

At least two species of lady beetles, the twice-stabbed laby beetle and the pitiful lady beetle, prey upon the gloomy scale to a considerable extent. The twice-stabbed lady beetle is much larger and more conspicuous than the pitiful lady beetle, but the latter appears to do more towards keeping the gloomy scale in control.

Another group of enemies of the gloomy scale are certain predaceous mites. These mites seem to be more scavengers than predaceous enemies, but on occasions they have been observed attacking the living scale insects.

Still another enemy of the gloomy scale is the so-called red-headed fungus. This disease is always apparent on trees that are badly infested with the gloomy scale, and not infrequently it is accused of being the real source of the trees' troubles. This disease does not appear to be of much value in checking the ravages of the gloomy scale, for it seems to attack the old exhausted females principally. It is usually more prevalent on the trunk and larger branches of the tree, where the scale insect cannot do as much damage as they do on the smaller branches.

CONTROL

Preventatives. Obviously the preventative to be used against the gloomy scale is to plant trees that are not usually attacked by this insect. The hard maples and the oaks are good shade trees and highly resistant to this pest and to most others. Objections are usually raised to these trees, however, because they grow so much slower than the soft maples; but when we take into consideration the effects of these insects, little seems to be gained, for the persistent attacks of the gloomy scale usually stunt soft maples so much that in the long run they do not make as satisfactory growth as the hard maples (Fig. 4). When we take into consideration, also, that the soft maples are more subject to other diseases, and that they are also more subject to damage during storms, and that limbs are more apt to be split off by heavy snows, we can readily see that there is very little use to plant soft maples. Since nurserymen are not always careful to send hard maples when they are ordered, the bud characters of the common hard and soft maples are shown in Fig. 14. By comparing the end buds of maple trees before they are set with these figures, any one can determine whether a maple is a hard maple or a soft maple, and all soft maples can be discarded.

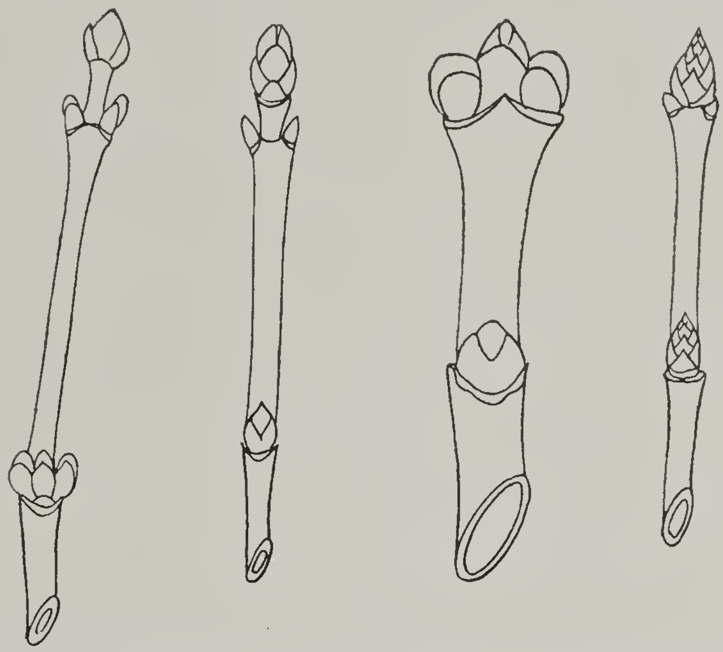

FIG. 14. Terminal buds of hard and soft maples.
A, Red maple; *B*, Silver maple; *C*, Norway maple; *D*, Sugar maple.

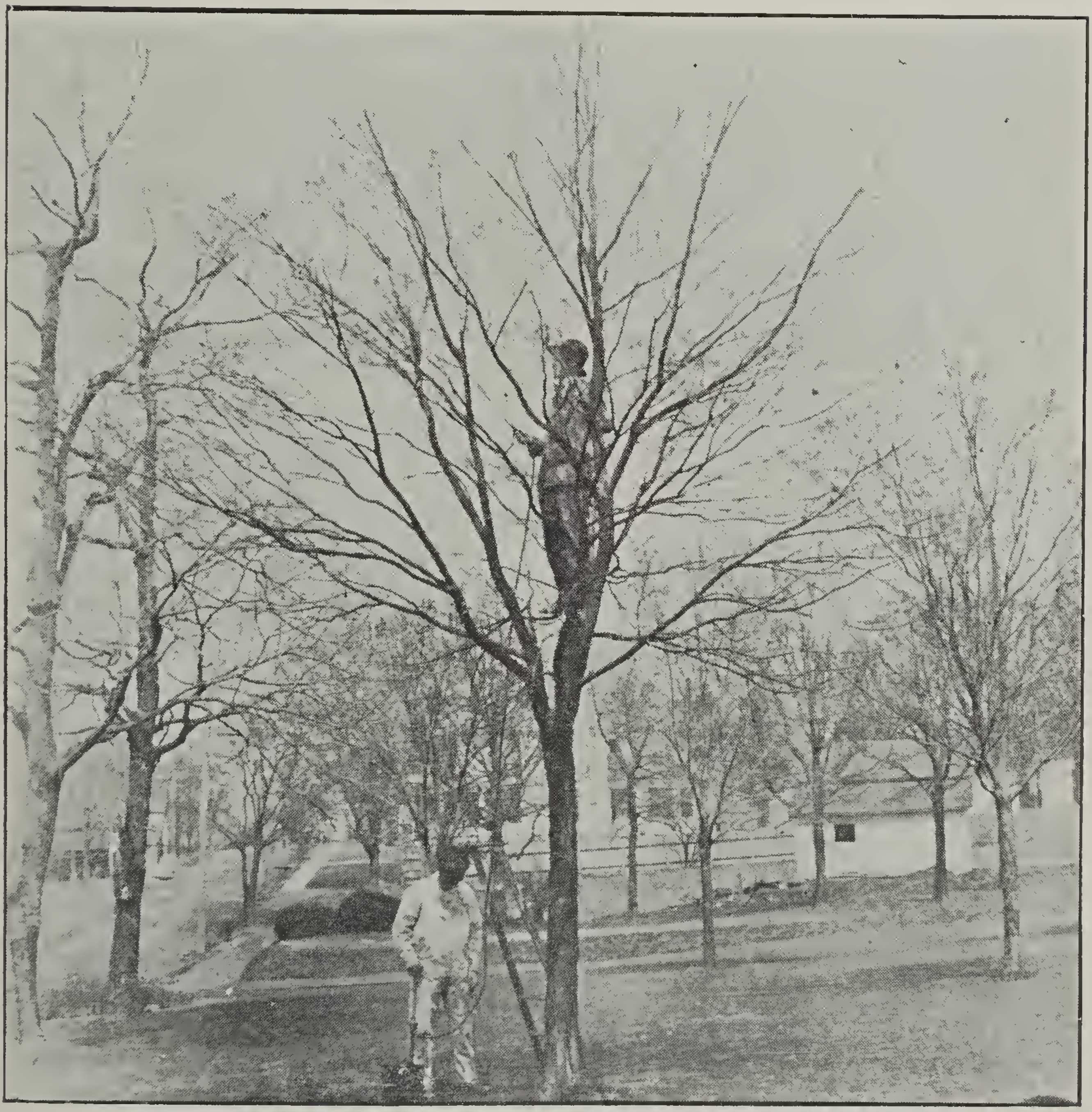

FIG. 15. A method of spraying small trees with a small sprayer.

Remedies. The standard remedy for the gloomy scale is to spray the trees thoroughly (Fig. 15) once each year, either late in the winter or early in the spring, with a mixture composed of one gallon soluble oil to eight to ten gallons of water. The soluble oils, which are sold under a variety of trade names, are heavy oils that make a milky emulsion when mixed with water. This mixture when sprayed on the trees kills only the scale insects that it touches, hence it is necessary that spraying of this kind be done very thoroughly and that care be taken to cover every limb and branch. Care must also be taken not to get any more of the spray mixture on the trees than is absolutely necessary, for oil injury has been reported on sprayed maples in other states, although it has never been noted in this State. The number of times it will be necessary to spray maple trees will depend upon a number of factors, chief among which are the amount of infestation and the care with which the spraying is done. Obviously a tree that is heavily infested will require more spraying than a tree that is only lightly infested. On the other hand, one careful spraying is the equal of two or more careless sprayings. Hence we set a standard of one spraying each year, and departures can be made from this standard as the case seems to demand.

Sprayings with strong oil solutions can be made only during the dormant life of the tree, that is, from the time the leaves are shed in the fall until the buds commence to swell in the spring. Apparently, scale insects are more susceptible early in the spring just at the time the buds are commencing to swell, and this is the most effective time for spraying. Summer sprayings are not successful against this pest because mixtures strong enough to kill the adults cannot be used without injuring the trees and the young are produced over such a long period that no remedy can be used against them successfully. Scraping, painting or whitewashing or scrubbing the trunks and larger limbs of infested trees is of very doubtful value, for the insects occur on the smaller limbs and branches in such numbers as to seriously damage the trees.

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JUNE, 1923 **TECHNICAL BULLETIN No. 22**

Digestive Coefficients of Poultry Feeds and Rapidity of Digestion

and

Fate of Grit in the Fowl

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Conducted jointly by the State Deparment of Agriculture and
the North Carolina State College of Agriculture and Engineering

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THE DIGESTIVE COEFFICIENTS OF POULTRY FEEDS AND RAPIDITY OF DIGESTION

AND

FATE OF GRIT IN THE FOWL

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HISTORICAL REVIEW

The absence of definite standards and coefficients of digestibility of different feeds for poultry has been a serious drawback to the best work of practical feeding tests. The alimentary canal of birds is quite different from that of mammals and it is desirable to determine if there is any difference in digestive coefficients between fowls and mammals. The fact that the undigested parts of the food and the urinary excretions are eliminated from the body together makes a serious obstacle in the way of protein digestion determinations with fowls and greatly increases the amount of analytical work to be done.

Some digestive experiments with poultry were done by Bartlett[1]. The length of time of feeding in these experiments was twelve to fourteen days, being divided into two periods, a preliminary period of five to seven days, when the amount the bird would eat was determined and the alimentary canal freed from other food, and a collection period. The droppings were collected in a specially constructed bag attached to the posterior parts of the bird and held in place by a kind of harness. The feeding was done at regular hours morning and evening. The droppings were collected at the same time and placed in glass jars and kept covered with alcohol to prevent fermentation. Later the alcohol was evaporated off on a steam bath, then dried at 60 degrees C. to air dry conditions.

Bartlett concludes after conducting his feeding experiments with different rations that "the indications are that the digestive coefficient of most nutrients for poultry are not materially different from other animals". "Unlike ruminants they digest very little crude fiber, hence coarser fodder carrying much of this material is of but little value. The nutrients of wheat do not seem to be as well handled as most other concentrated grains, the ether extract showing a particularly low coefficient. Likewise bran, one of the wheat by-products, shows low digestibility in organic matter including ether extract and nitrogen-free-extract. Its bulky and coarse condition has brought it much into favor to feed with more concentrated materials and it often makes as much as fifty per cent of the mixture used in mashes."

"The mixture of equal parts corn meal and fine cut early clover is sufficiently bulky to feed with concentrates and is more digestible. An exclusive diet of wheat has a deranging effect upon the digestive system of the birds." He further concludes that corn is the most valuable grain for poultry, being high in digestibility and palatibility.

The feeds tested were wheat bran, corn meal, gluten feed, beef scrap, linseed meal, corn, cracked corn, cut clover, oats, rolled oats, wheat both hard and soft.

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[1] Bartlett, J. M., Digestive Experiments with Poultry, Bul. 184, Me. Exp. Sta., 1910.

Corn showed a higher digestibility than any other grain tested.

Wheat bran showed a low digestibility.

A mixture of equal parts fine cut clover and corn meal is more digestible and is a more economical feed than bran.

Corn compares favorably with oats as a grain feed for fowls. The ether extract of wheat has a low digestive coefficient.

Crude fiber is but very little digested and evidently is of but little use in a ration for poultry except to give bulkiness.

The mixing of about seven per cent of bone ash with a ration consisting wholly of vegetable matter gave slightly higher average digestive coefficeint than when the mixture was fed without it.

Kalugine[2] in 1897 conducted digestion trials with peas, buckwheat, wheat, and barley. "Hens digest the crude protein of peas and barley in about the same proportions as has been observed in experiments with farm animals. The assimilation of crude protein of wheat and buckwheat is inferior to that found in the other two grains. In their capacity to utilize fat, fowls in some respects resemble swine and in other respects herbivora.

Fields and Ford[3] in 1900 conducted digestion trials with kafir corn, corn, and cowpeas. The digestion coefficients of kafir corn and corn are higher when fed whole than when ground. The reverse is true of cowpeas. The percent of digested crude protein, nitrogen-free-extract, and ether extract is generally higher in kafir corn than in cowpeas.

Lehmann[4] conducted trials in 1901 with wheat and peas. Fiber was found indigestible. The assimiliation of both crude protein and fat of wheat is much less than that of peas. The reverse is true of nitrogen-free-extract. The digestible coefficients by poultry are more comparable to those of swine than to those by farm animals.

Paraschtschuk[5] in 1902 conducted trials with corn for hens and cocks. "Digestion by poultry does not differ widely from that of other animals. In their ability to digest fiber, poultry resembles swine more than they do ruminants or horses. In their ability to digest ether extract poultry most closely resembles ruminants. They are lowest in order in ability to utilize nitrogen-free-extract."

Prior to 1904 digestion trials were reported for about 20 feeds on poultry. Duplicate trials were reported for only three feeds. These are corn, peas, and barley.

Brown[6] in 1904 gives the results of sixteen digestion trials with nine hens and one cock extending over a period varying from six to thirteen days. The feeds used were corn, oats, wheat, and meat. Crude protein and nitrogen-free-extract are assimilated in much greater proportions in corn than in oats. Wheat falls between the two in these respects. The digestibility of crude fat of wheat is conspicuously less than of corn and oats, which fact may bear relation to the unfavorable results that follow sole wheat diet. Chickens eat much more of corn than of oats and the nutritive superiority of corn is manifest in an increased body weight of these chickens in the corn test in contrast with a decreased body weight of chickens in the oat test. According to the availability of the nutrients, these three grains vary with respect to low cost in order of corn, oats, and wheat.

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[2] Kalugin, J. Ueber die Wirkung fiene Grandes awf die verdanlich keit der Nohrstoffe der Hirse bei Huhnern. Fuhling's laudwirtsch. Zlg. Leipzip, 46, Heft 3, I Feb. Pp 85-86, 1897.

[3] Fields and Ford, Bul. 46, Okla. Exp. Sta., 1900.

[4] Lehmann, F. Futterungsveruche beitr. Ernahrung von Geflugee. Deutschcland wirtsch. Gresse Berlin, 28 J., No. 39, 118, Mar. pp 339-340, 1901.

[5] Paraschtschuk, Simon. Die Verdanung des mais hei Huhnern, Journ f. Zandwirt Sch., Berlin 50. J., Hebt 1, 9 mai, pp 15-32, 1902.

[6] Brown, E. W., Bul. 56, U. S. Dept. of Agri., B. A. I. Digestion Experiments with Poultry, 1904.

The digestive organs of the fowl differ greatly from that of mammals[7]. The food passes from the mouth through the pharynx and first portion of the esophagus to the crop. The food here becomes moistened, then passes through the second portion of the esophagus to the proventriculus where it is soaked in a strong acid solution. It then passes into the gizzard and is ground. Thus ground and soaked with acid and a ferment pepsin it passes to the duodenum where pepsin digestion is completed. The content of the duodenum, about fourteen inches long, is strongly acid. At the lower end of the duodenum the bile and pancreatic secretion is poured out and the reaction gradually becomes less acid till the caeca, two in number, are reached. The liquid flowing into the large intestine, which in the averaged sized hen is about four and one-half inches long, now by reverse peristalsis of this portion forces the liquid forward, at the same time the caecal valves extend across the mouth of the free portion of the small intestine and the content is thus forced into the caeca. This can be demonstrated by forcing liquid through a syringe into the large intestine.

Since the urine and the undigested food accumulate in the cloaca and are voided together in the fowl, a means must be used in determining the uric acid content in analyses of the excreta of fowls, a factor not present in digestion work in mammals since in mammals the urine is eliminated from the body through another channel from the undigested food channel or bowel.

Avian urine has a relatively small water content which rapidly evaporates and leaves a white flaky mass of uric acid so commonly seen in the droppings of birds. The urea, which makes up the major part of the nitrogenous end-products of mammalian urine, is highly soluble, whereas the uric acid making up the bulk of the nitrogenous end-product in avian urine, is relatively insoluble. The insolubility of the uric acid is an important factor in making the uric acid determinations and complicates the test. Mammalian urine is rich in chlorides, phosphates, sulphates, calcium, and magnesium. Avian urine contains relatively none of these. The avenue of escape for the salts must be in another direction and it is interesting to note that the yolk and shell of the egg contains considerable quantities of such salts.

From a review of literature at hand on this subject it would appear that fowls are apparently most efficient in digesting the nutrients in the order of nitrogen-free-extract, protein, and fat. They digest relatively little or no fiber.

THE PROBLEM

The purpose of the work is to determine the rapidity of digestive processes in the fowl and to make sufficient digestive coefficient studies of poultry feeds which, together with those already available, would be sufficient for a tentative table of digestible nutrients of poultry feeds based on digestion experiments with poultry, to determine the ash intake and ash outgo and its effect on the digestive coefficients of feeding stuffs of poultry, and to determine the fate of grit in the fowl.

Time Required for Food to Pass Through the Entire Intestinal Tract of Fowls

Fowls of the American breeds were used in these experiments. Only hens were to be tested, and birds two to three years of age were selected.

The birds were kept in small wire coops eighteen inches square with one inch chicken netting floor. The coop stood on a tin pan slightly larger than the floor of the coop. This pan was to catch the excreta as it was voided.

Ground feeds such as wheat middlings and corn meal were used in which was incorporated a material that would stain the digestive content and indicate just when the food passed out of the body. The materials, or indicators were lampblack, methylene blue, and gentian violet.

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[7] Kaupp, B. F., Anatomy of the Domestic Fowl, published by W. B. Saunders Co., Philadelphia, Pa., 1918.

DATA

Test No. 1: White Plymouth Rock hen, leg band No. B36, weight 5.9 pounds.

8 a. m. given 20 gms. wheat middlings mixed with water and lampblack.

2 p. m. first evacuation, a trace of lampblack appeared.

4 p. m. still only a trace of lampblack appears, fed 20 gms. wheat middlings without lampblack.

6 p. m. evacuation showing excreta decidedly black.

8 a. m. second day 20 gms. middlings and water given.

8 a. m. third day—48 hours—excreta showed slight tinge of lampblack on the outer surface but none on the inner particles. 20. gms. middlings mixed with water given. 6 p. m. 20 gms. middlings mixed with water given.

8 a. m. fourth day—72 hours—excreta normal. No trace of lampblack. Lampblack appeared in six hours and disappeared in seventy-two hours.

Test No. 2: White Plymouth Rock hen, leg band No. 142, weight 6.8 pounds.

8 a. m. given 20 gms. wheat middlings mixed with water, lampblack.

4. p. m. fed 20 gms. wheat middlings with water and lampblack.

6. p. m. first excreta passed. Lampblack stained excreta.

8 a. m. second day, 20 gms. middlings given.

6 p. m. 20 gms. middlings given. Excreta still black.

8 a. m. third day 20 gms. middlings given. Excreta nearly normal, only small amount on the outside, inside of mass not containing lampblack.

6 p. m. 20 grams middlings given.

8 a. m fourth day—72 hours—excreta normal.

In this case the first excreta voided after the feeding of the lampblack was at 6 p.m., or ten hours. At this time lampblack in small amounts appeared. All lampblack had disappeared at the end of 72 hours.

Test No. 3: White Plymouth Rock hen, leg band No. B10, weight, 6.14 pounds.

7 a. m. fed 10 gms. wheat middlings mixed with water and lampblack.

1 p. m. first evacuation. The excreta was black.

2 p. m. second evacuation. Excreta black.

7 a. m. fourth day. Excreta free from black.

Lampblack appeared in six hours and disappeared in seventy-two hours. This test was run with a broody hen.

Test No. 4: White Plymouth Rock hen, leg band No. 473, weight 5.14 pounds.

7 a. m. fed 10 gms. wheat middlings mixed with water and lampblack.

4 p. m. fed 20 gms. middlings, no lampblack. No excreta was voided on the first day after feeding with lampblack. Excreta showed black at 7 a. m. second day. Excreta were also voided at 7:30 second day which was also black. This bird acted as though constipated and no further excreta were voided until morning of the fourth day or seventy-two hours. No lampblack was present in these last excreta.

Owing to the fact that no excreta were voided on the day of feeding of the middlings and lampblack it is impossible to tell how long it required for the lampblack to pass the entire length of the digestive tract. The fact that the excreta were free from lampblack on the fourth day and after the seventy-two hours showed this test to be in line with those above. This test was run with a broody hen.

Test No. 5: White Plymouth Rock hen, leg band No. B148, weight 6 pounds.

7 a. m. fed 10 gms. wheat middlings mixed with water and lampblack.
4 p. m. fed 20 gms. middlings.
7 p. m. fed 20 gms. middlings.
9 p. m. first excreta voided. Excreta black.
7 a. m. fourth day excreta after 72 hours normal. No lampblack.

This test was run on a broody hen. First excreta voided in 14 hours and showed lampblack. Lampblack traces disappeared after 72 hours.

Test No. 6: Single Comb White Leghorn hen, laying condition, leg band No. 34, weight 2.8 pounds.

7 a. m. fed 20 gms. wheat middlings mixed with water and lampblack.
10:30 a. m. first excreta voided. Excreta black.
11:20 a. m. excreta voided, same color as above
7 a. m. fourth day after 72 hours excreta normal.

Excreta showed in three and one-half hours that food had passed the entire length of the intestinal tract.

Test No. 7: Chick, leg band No. 32, weight 2 pounds.

7 a. m. fed 10 gms. wheat middlings mixed with water and lampblack.
10:50 a. m. first excreta voided. Excreta black
7 a. m. fourth day after 72 hours excreta normal

The indicator suggests that the food passed through this two-pound broiler chick in four hours.

Test No. 8: Chick, leg band No. 36, weight 1.8 pounds.

7 a. m. 20 gms. wheat middlings given mixed with water and lampblack.
10:55 a. m. first excreta voided. Trace of lampblack.
11:40 a. m. second excreta voided. Excreta black.
7 a. m. fourth day after 72 hours excreta normal.

In this case the excreta voided indicated that the food had passed through the digestive tract in approximately four hours.

Test No. 9: Buff Plymouth Rock hen, in laying condition, leg band No. 26 weight 5.5 pounds.

7 a. m. fed 20 gms. wheat middlings mixed with water and lampblack.
10:00 a. m. first excreta voided. Excreta black.
7 a. m. fourth day, excreta normal.

The indicator showed that food had passed the entire digestive tract in three hours. An egg was laid first day of experiment.

Test No. 10: Buff Plymouth Rock hen in laying condition, leg band No. 28, weight 6 pounds.

7 a. m. fed 20 gms. wheat middlings with water and lampblack.
10:05 a. m. first excreta voided. Excreta black.
7 a. m. fourth day after 72 hours excreta normal.

The indicator showed that food passed through the intestinal tract in approximately three hours. This hen laid one egg on the first day of the experiment.

Test No. 11: Buff Plymouth Rock hen, in laying condition, leg band No. 30, weight 6 pounds.

7 a. m. fed 20 gms. wheat middlings mixed with water and lampblack.
10:00 a. m. first excreta voided. Lampblack present.
3:30 p. m. third day all traces of lampblack gone.

The hen laid an egg each of the first two days. The indicator showed that food had passed the entire length of the digestive tract in approximately three hours. It was noted that in the latter part of this experiment that the outside of the fecal mass was black and the inside not colored. This raised the question as to whether lampblack will adhere to the intestinal mucosa and be gradually eliminated by the feces as it passes along. In the small intestines where the food is mixed by the pouring back and forth process, it evidently would be mixed with the food. However, this would not hold true in the large intestine, since the formation of the mass takes place either in the large intestine or cloaca or in both.

With these ideas in mind it was decided to try some test using aniline blue dyes which were calculated to stain the feeds and not be in a form to be carried along mechanically as in the case of lampblack.

Test No. 12: Columbian Wyandotte hen, leg band No. 26, weight 5.4 pounds.

7 a. m. fed whole corn soaked in gentian violet water. She ate 5 gms.

4 p. m. given 20 gms. soaked in gentian violet water.

7:30 a. m. second day, first evacuation. Excreta watery in consistency, greenish in color, indicating at first a tendency to constipation and later diarrhea. This test was considered unsatisfactory.

Test No. 13: Columbian Wyandotte hen, leg band No. 28, weight 5.6 pounds.

7 a. m. fed whole corn soaked in gentian violet water. She ate 4 gms. There was no evacuation during the day.

8:45 a. m. second day, first evacuation watery in consistency, greenish in color. This test was unsatisfactory for the same reason as No. 12.

Test No. 14: Columbian Wyandotte hen, leg band No. 31. weight 6 pounds.

7 a. m. fed whole corn soaked in gentian violet water. She ate 3 gms.

6 p. m. first evacuation. Greenish and water-like in consistency. The constipating effect was not so great as in the two previous cases. This test was considered unfavorable and unsatisfactory.

Test No. 15: Columbian Wyandotte hen, leg band No. 32, weight 5.7 pounds.

7 a. m. fed whole corn soaked in gentian violet water. She ate 10 gms.

9 a. m. second day, first evacuation. Feces greenish and watery. The hen appeared weak. Here again the gentian violet appeared to cause constipation followed by diarrhea apparently due to irritation of the mucosa of the bowel. This test was considered unsatisfactory.

Test No. 16: Buff Plymouth Rock hen, leg band No. 26, weight 5.8 pounds.

8:30 a. m. fed corn meal mixed with methylene blue water.

11:40 a. m. first excreta voided. The excreta were blue at this time.

7 a. m. fourth day after 72 hours excreta normal.

This was three hours and ten minutes for food to pass through the intestinal tract. There was some irritation. This bird was in laying condition.

Test No. 17: Buff Plymouth Rock hen, leg band No. 28, weight 6 pounds.

8:30 a. m. fed 35 gms. corn meal mixed with methylene blue water.

11:00 a. am. first excreta voided. Excreta were blue. There was some irritation at this time evidence by slight watery condition of the excreta.

8:30 a. m. fourth day after 72 hours all traces of the blue had disappeared.

Food passed through the digestive tract in two hours and thirty minutes. This bird was in laying condition. There was here again evidence of irritation.

Test No. 18: Buff Plymouth Rock hen, leg band No. 30, weight 5.9 pounds.

8:30 a. m. fed 8 gms. corn meal mixed with methylene blue water.

9:00 a. m. first excreta were voided. Only very slight traces of methylene blue could be detected.

4:30 p. m. second evacuation. Excreta blue. The first passage was in thirty minutes and indicated that if food is given on an empty crop and mixed with methylene blue slight staining of the food in the cloaca may occur in that space of time. The reaction was constipation followed by looseness of the bowels as with the gentian trials. At the end of 72 hours no trace of the methylene blue could be detected.

Test No. 19: Buff Plymouth Rock hen, leg band No. 32, weight 5.7 pounds.
8:30 a. m. fed 30 gms. corn meal mixed with methylene blue water.
11.40 a. m. first excreta were passed. Excreta blue.
8:30 fourth day, after 72 hours excreta normal.

The first indication was at three hours and ten minutes. There was again evidence of irritation. The hen was in laying condition, having laid two eggs during the trials.

Test No. 20: Buff Plymouth Rock hen, leg band No. 34, weight 6.1 pounds.
8:30 a. m. fed 31 gms. corn meal mixed with methylene blue water.
12:00 m. first excreta passed. Excreta blue.
8:30 a. m. fourth day, after 72 hours excreta normal.

Indicator showed food passed entire digestive tract in three hours and thirty minutes. Hen was in laying condition. Methylene blue caused irritation.

Test No. 21: Buff Plymouth Rock hen, leg band No. 36, weight 6.3 pounds.
7 a. m. fed 7 cc. methylene blue water mixed with corn meal.
7:30 a. m. second day, first evacuation. Excreta blue.
7:30 a. m. third day, after 48 hours all blue had disappeared.

This bird was not in laying condition. The first effect was that of constipation, the first evacuation being twenty-three hours after the test meal was given.

Test No. 22: White Plymouth Rock hen, leg band No. 115, weight 6.5 pounds.
7 a. m. fed 20 gms. corn meal mixed with lampblack.
10:20 a. m. first excreta voided. Excreta black.
7 a. m. fourth day, after 72 hours excreta normal.

This hen was in laying condition, laying an egg on each of the three days of the test. The food, as indicated by the lampblack, passed the entire length of the digestive tract in three hours and twenty minutes. The hen was in laying condition.

Test No. 23: White Plymouth Rock hen, leg band No. 56, weight 5.8 pounds.
7 a. m. fed 20 gms. corn meal in which was incorporated lampblack.
12:20 p. m. first excreta were voided. Excreta black.
7 a. m. fourth day, after 72 hours excreta normal.

The first colored excreta were passed in five hours and twenty minutes. The hen was in laying condition laying each day during the test.

Test No. 24: White Plymouth Rock hen, leg band No. 100, weight 6.3 pounds.
7 a. m. fed 20 gms. corn meal mixed with lampblack.
2 p. m. first excreta passed. Excreta black.
7 a. m. fourth day, excreta normal.

The first excreta were voided in seven hours. This hen was broody. Broody hens apparently do not have as many evacuations as other hens and the quantity evacuated is greater as indicated by this hen.

Test No. 25: Partridge Plymouth Rock hen, leg band No 112, weight 5.3 pounds.
7 a. m. fed 20 gms. corn meal in which was incorporated lampblack.
1:20 p. m. first excreta voided. Excreta black.
7 a. m. fourth day, after 72 hours excreta normal.

The first excreta indicating lampblack was voided in six hours and twenty minutes.

DISCUSSION

Of the two White Plymouth Rock hens not in laying condition one passed wheat middlings in six hours and the other in ten hours or an average of eight hours.

In the test with one Single Comb White Leghorn in laying condition the wheat middlings passed through the intestinal tract in three and one-half hours. Of the three Buff Plymouth Rock hens in laying condition, two passed wheat middlings in three hours and the third in three hours and five minutes. Of the two White Plymouth Rock hens laying during the tests, corn meal passed through the digestive tract, one in three hours and twenty minutes and the other in five hours and twenty minutes, or an average for these two of four hours and twenty minutes. An average of the six tests with laying hens showed that food passed the entire digestive tract in three hours and forty-six minutes.

Of the three White Plymouth Rock hens that were broody, one passed the wheat middlings through the intestinal tract in six hours, the second in twenty-four hours, and the third in fourteen hours, or an average of these three of fourteen and two-thirds hours. Of two White Plymouth Rock hens in a broody condition given corn meal one passed food through the digestive tract in seven hours and the second in six hours and twenty minutes, or an average of the five broody hens of eleven hours and forty-five minutes.

Of the two chicks weighing approximately two pounds each, one passed the wheat middlings through the intestinal tract in three hours and fifty minutes and the other in three hours and fifty-five minutes, making an average of three hours and fifty-two minutes.

Of the four Columbian Wyandotte hens not in laying condition, one consumed 5 gms. whole corn soaked in gentian violet water and voided first excrement in twenty-four hours, the second consumed 4 gms. and passed the first tinted excreta in 25 hours, the third ate 3 gms. and passed the first tinted excreta in twenty-six hours. From this it is evident that gentian violet causes constipation followed by a looseness of the bowels, indicating irritation to the mucosa of the bowels.

Of six Buff Plymouth Rock hens in laying condition, two consumed 30 gms. whole corn soaked in methylene blue water, and the first tinted excreta was voided in three hours and ten minutes. One consumed thirty-five gms. and voided tinted excreta in two hours and thirty minutes, the fourth consumed thirty-one grams corn meal and voided tinted excreta in three hours and thirty minutes, the fifth consumed 8 gms. and voided the first tinted excreta in one hour and thirty minutes, the sixth consumed 7 gms. and voided the first tinted excreta in twenty-three hours. The tendency here is for small amounts of methylene blue to cause constipation and larger doses irritation with specks of blood on the semi-liquid evacuations. There is only one exception to this tendency and that is in the case of the bird that consumed 8 gms. of corn and evacuated in one hour and thirty minutes.

SUMMARY

Digestive processes of the fowl are rapid. The greatest rapidity is shown in the laying and in the growing fowl, food passing on an average of three hours and fifty two minutes in the case of growing fowls and three hours and forty-six minutes in the cases of the laying hens. Next in activity comes the adult hen not in laying condition, averaging eight hours, and then the broody hen required an average of eleven hours and forty-four minutes.

It was noted that broody hens behaved the same in the experimental coops as on the nest, that is, the evacuations were fewer and the quantity evacuated each time increased over that of a normal hen.

We have not taken as accurate the fowls in which the gentian violet or methylene blue were given, as it exercised influence on the normal function of the intestinal tract.

In all these tests the hens were placed in the coops the day before the trial so that the crop was empty when the test feed was given.

EXPERIMENTAL FEEDING METHODS

The following method of feeding was used throughout our work. The coop as shown in Fig. 5 is made of four upright 2″ x 2″ pieces. The coop is eighteen inches square, the bottom of which is made of one inch mesh chicken netting and is two inches above the pan in which the coop is placed. The side of the coop is constructed of one inch chicken netting. The top is removable and is made of four 1″ x 2″ wooden strips nailed to two pieces of 1″ x 2″ at right angles. The dropping pan is constructed of block tin twenty inches square and one inch deep. It is reinforced at the rim with heavy copper wire.

The receptacle used for feeding consisted of a large, long-handled dipper in the bottom of which was soldered a 250 cc. tin cup. In the cup was placed the feed which was being used in the experiment. The hen stood in such a position when eating that if any of the feed was thrown outside the cup, it remained inside the large dipper and did not become wasted or mixed with the excreta. The correction was properly made in the feeding data.

The feeding experiments were conducted in a well-ventilated and well-lighted basement room. The feeding coops were placed on a specially constructed white top table and arranged in such a manner that if by any reason either feed or excreta were thrown out of the proper receptacles, it could at once be seen and proper corrections made.

In selecting our fowls we chose mature hens of lymphatic temperament. Such hens were brought from the Station and College poultry plant in the morning and placed in the feeding coops and allowed to remain till the next morning, being given only water. This allows the intestinal tract to become partially empty and conserves the appetite, thereby making the bird more fitted for the experiment.

A definite number of grams of the test feed was given at 7:30 a. m. and 4:30 p. m. The feed used in the experiment was always mixed with an equal number of grams of water and placed before the fowl in the feeding cup. It was soon observed that the bird would consume all the feed she cared to in twenty minutes.

The digestion experiment was divided into two periods, the first a four day preliminary period, the second a four day test period. Accurate records of feed consumed during these two periods were kept as shown by records of the appended tables. Excrement for analyses was saved only from the test period.

CARE OF EXCREMENT

Each morning preceding the feeding all excrement that remained on the wire bottom coop was carefully removed with a spatula and placed in the receiving pan. These pans were removed and replaced by clean pans.

The excrement was removed from the pan with a spatula and placed in a 500 cc. procelain dish. The excrement that could not be removed with a spatula was washed off with alcohol and this washing was also placed in the porcelain dish. The excrement in the dish was then covered with 95% alcohol to prevent fermentation; to which 5 cc. of acetic acid was then added to convert the free ammonia into ammonium acetate. This was then dried as thoroughly as possible on a water bath. After drying on the water bath it was then placed in an electric oven and held at a temperature of 110 degrees C. for five hours. It was then removed and carefully weighed and placed in an air-tight container and properly labeled for analysis.

ANALYSES OF FEEDS

Moisture

Two samples of about three grains each of the substance is put into weighing bottles with ground glass covers. These are put into an electric oven and heated for five hours at 110 degrees C. After heating the bottles are allowed to cool, then weighed, heated again, and reweighed. This is continued until the weight is constant to 2 mg. From the loss of weight the percentage of dry matter is calculated and the percent of moisture is determined

Ether Extract

A moisture-free sample is taken, representing about two or three grams of the substance. This is put into an extraction thimble. This thimble is placed inside of a soxhlet extraction siphon that is then connected to a Liebig condenser. This is then connected to a weighed extraction flask or bulb containing about 75 cc. of anhydrous ether. The extraction is continued for about two hours in an electric oven air bath.

The extracting flask or bulb is then put in a still and the excess of ether is recovered. The extraction flask is put into the electric oven and dried for one hour at 100 degrees C., removed from the oven and placed in a desiccator, cooled and weighed. This process of heating and weighing is continued until the minimum weight of fat is calculated.

Crude Fiber

The residue from the ether extract determination is put into a 500 cc. flask to which is added 200 cc. of boiling 1.25 percent of H_2SO_4. This is connected to a condenser and boiled continuously for thirty minutes. This is filtered through a linen filter and washed with boiling water until the washings are no longer acid. The residue is then put back into the flask with 200 cc. of boiling 1.25 percent N_aOH free as possible from sodium carbonate, and is boiled continuously for thirty minutes in the same manner as given for boiling with the acid. The alkaline solution is filtered at once and washed as rapidly as possible, and continued until the washings are neutral. The residue is dried at 110 degrees C. until it ceases to lose weight, weighed, incinerated completely, and weighed again. The loss of weight is considered to be the crude fiber.

Total Proteins

A sample of 0.7 grams of the substance is put into a Kjeldahl digestion flask, to which is added 10 gms. of powdered potassium sulphate, 0.7 gm. mercuric oxide, and 25 cc. H_2SO_4. This mixture is then subjected to a slow heat for five minutes and a high heat for twenty minutes, or until digestion is complete. This is done under a hood. The liquid is then allowed to cool and to it is added 200 cc. of nitrogen-free water and a few pieces of copper wire. The flask is taken to the Kjeldahl distilling apparatus and 50 cc. of a saturated sodium hydroxid and 20 cc. of a potassium sulphate solution is added. The apparatus is then connected to the condenser. The content of the flask is mixed by shaking. One hundred fifty cc. are then distilled into a standard acid solution. The standard acid solution is then titrated and the percent nitrogen determined and this multiplied by 6.25 gives the amount of protein.

Ash

Two grams of the substance is weighed into a crucible, burned at a slow heat for twenty minutes, then fast heat until all of the carbon is burned, and a constant weight is maintained. Then the percent of ash is determined.

Nitrogen-Free-Extract

The nitrogen-free-extract is determined by difference.

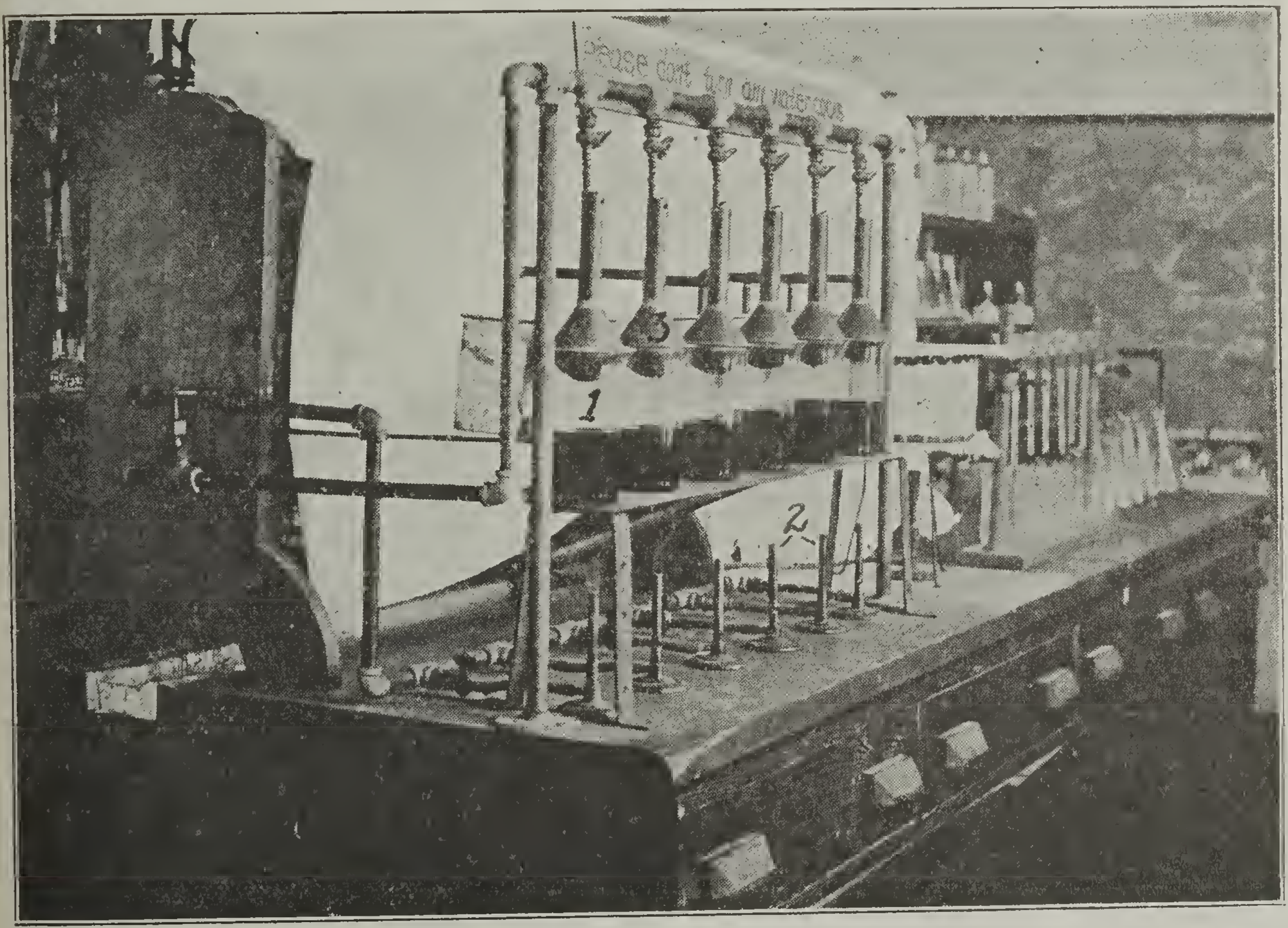
Please don't turn on water
3
1
2

FIG. 1. CRUDE FIBER DETERMINATION APPARATUS

1. Beakers containing the feed sample.
2. The bunsen burners.
3. Copper water bulbs through which water circulates to prevent boiling over.

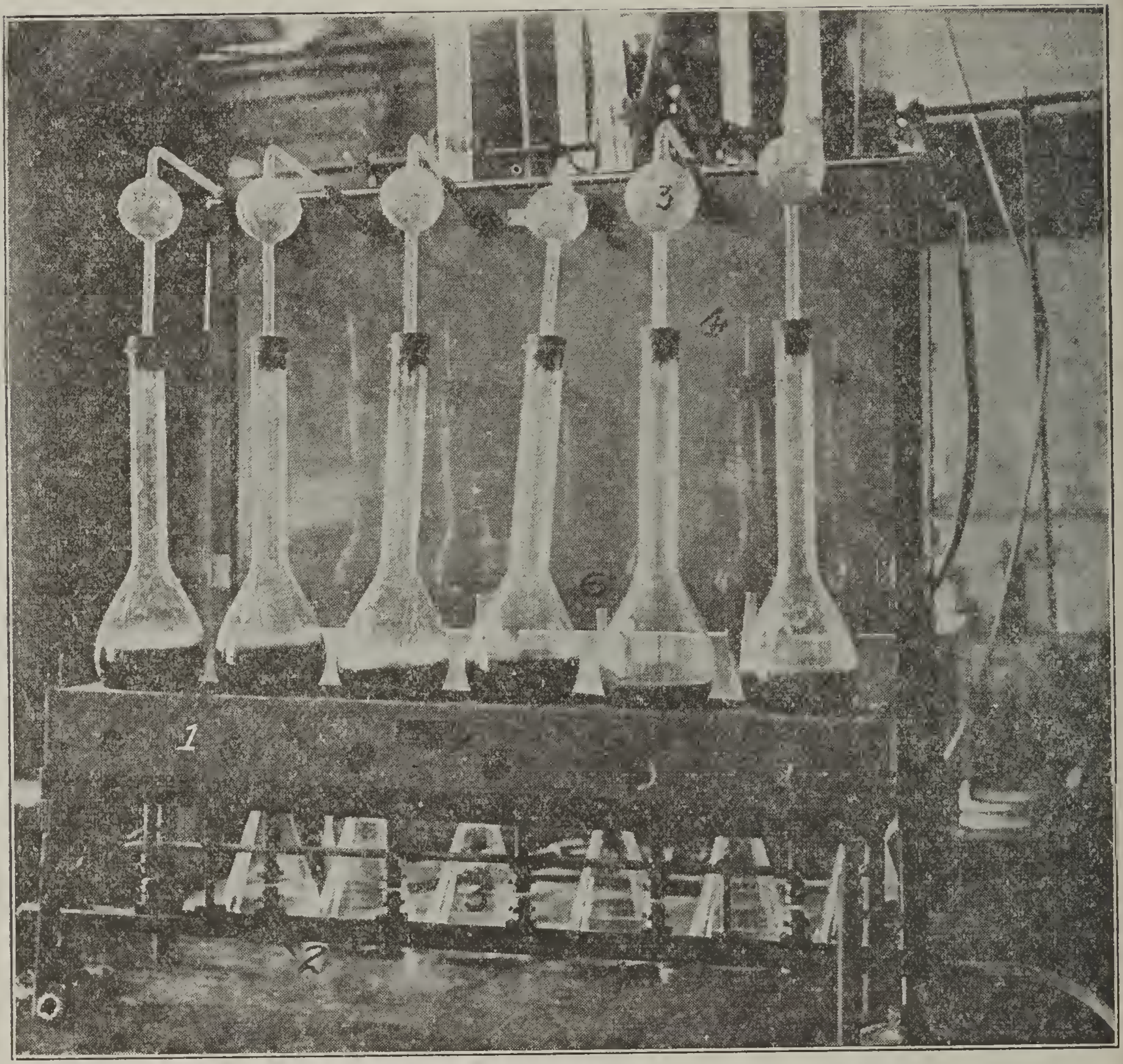

FIG. 2. KJELDAHL DISTILLING APPARATUS FOR THE DETERMINATION OF NITROGEN

1. Rack supporting Kjeldahl flasks.
2. Showing beneath it bunsen burners and gas connections.
3. Glass bulbs preventing direct overflow of boiling liquid in Kjeldahl flask.
4. Condenser with lead coils inside surrounded by running water. From the lead coils the condensed liquid flows into Erlingmeyer flasks 5, 6 expanded tube inserted in the stopper of the receiving Erlengmeyer flask containing glass beads moistened with water to prevent the escape of free nitrogen during the process of distillation.

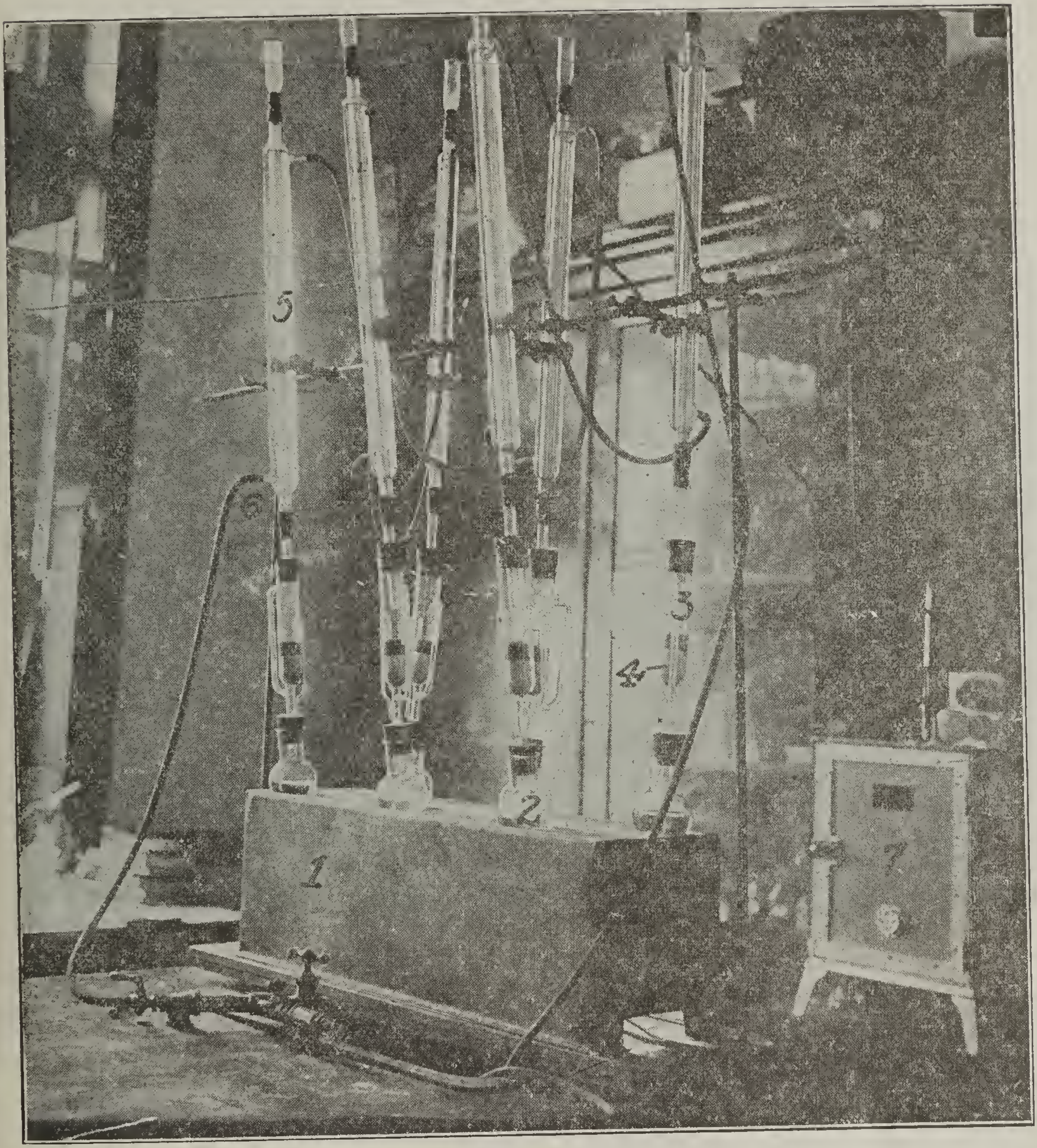

Fig. 3. Apparatus for Determining the Ether Extract

1. Metal box having within six 120 watt nitrogen bulbs forming an electric air bath for distillation of ether.
2. Fat extraction flask receiving the extracted fat.
3. Soxhlet extraction tube containing extraction thimble.
4. Thimble containing the material from which to have the fat extracted.
5. Liebig's condenser showing rubber connections at 6 for inlet for cold water.
7. Electric oven for drying fat.

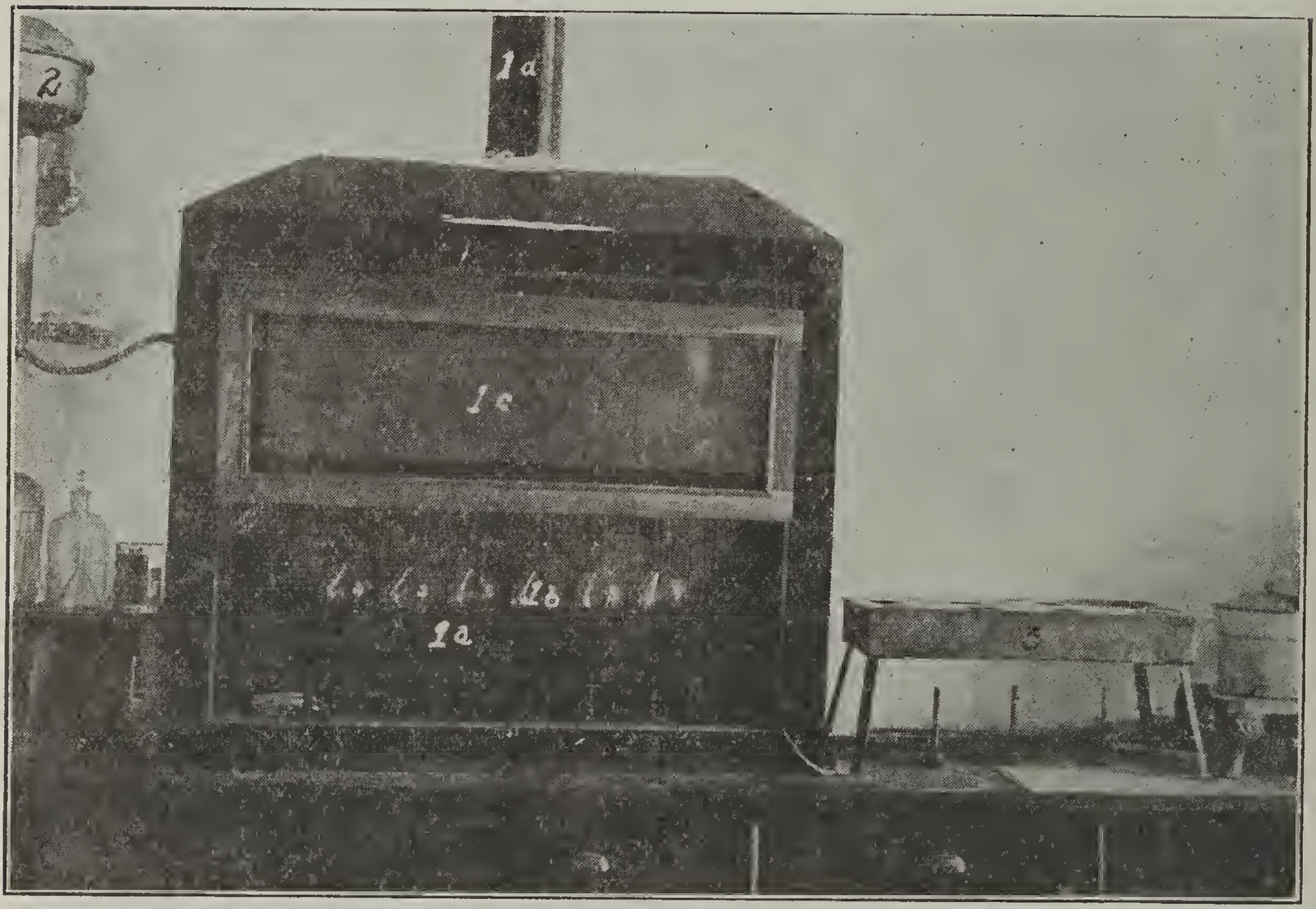

FIG. 4. PHOTOGRAPH OF A KJELDAHL DIGESTION SHELF USED IN THE EXPERIMENTAL WORK

1a. The shelf; 1b. The digestion flasks; 1c. The glass door to hood; 1d. flue to conduct the gas out of the hood.
2. The water still used to produce the distilled water.
3. The water or steam bath used in evaporation of excrements.

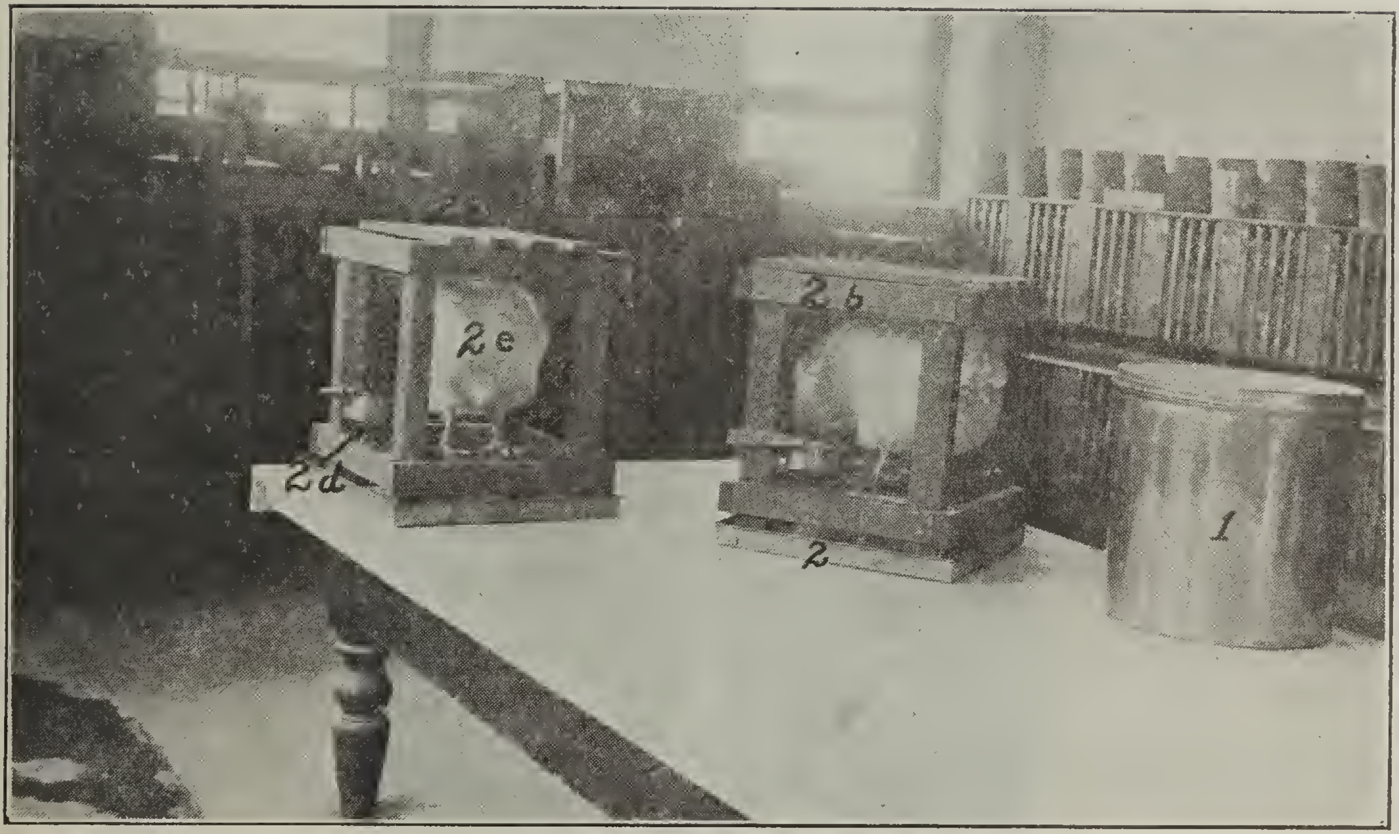

Fig. 5. Photograph Showing Methods of Handling Birds in Feeding Tests

1. Feed container; 2b. Feeding crate; 2d. The feed cup.
2. Droppings pan; 2c. Removable slatted top; 2e. The experimental hen.

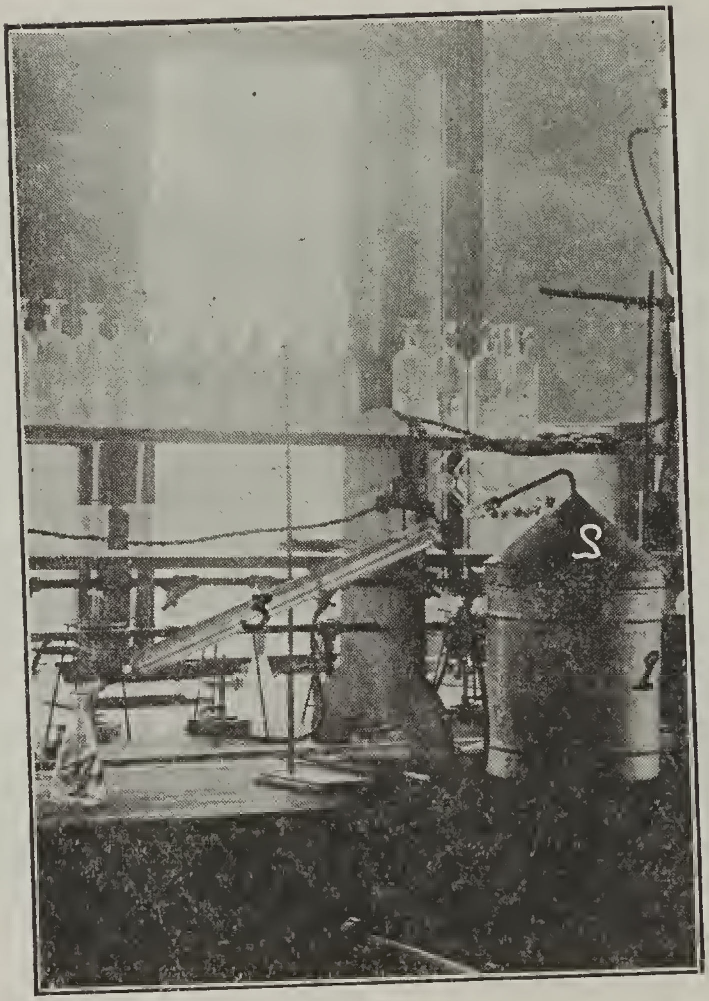

FIG. 7. APPARATUS FOR THE RECOVERY OF ETHER

1. Body of evaporator in which is placed a 120 watt nitrogen bulb.
2. Hood in which the fat bulbs containing fat and ether are placed.
3. Condenser.
4. Receiving flask.

GROSS AND CHEMICAL ANALYSES OF THE EXCRETA

The droppings were reduced to fineness by grinding or by beating in a large iron mortar with a large iron pestle. The material was then passed through a wire sieve with 256 meshes to the square inch.

In a gross study of this material there was observed grit consisting of quartz or silica, varying in size up to 2 x 4.5 m.m. and weighing 0.0813 gm.

Black bodies were also observed and under the hand lens they were recognized as pieces of cinders. One piece of glass was observed in one study. These birds had not been given grit for the entire period they were used. This includes the four preliminary days and the four test days in which the droppings were saved. Other bodies recognized included undigested feed. Chalk-like bodies, the largest of which measured 2 x 3 x 4 mm. and weighed 0.0112 gm, consisted of urinary sediment and mostly of uric acid. In three tests the average weight of coarse parts was 0.0931. The percentage of foreign matter was 0.56.

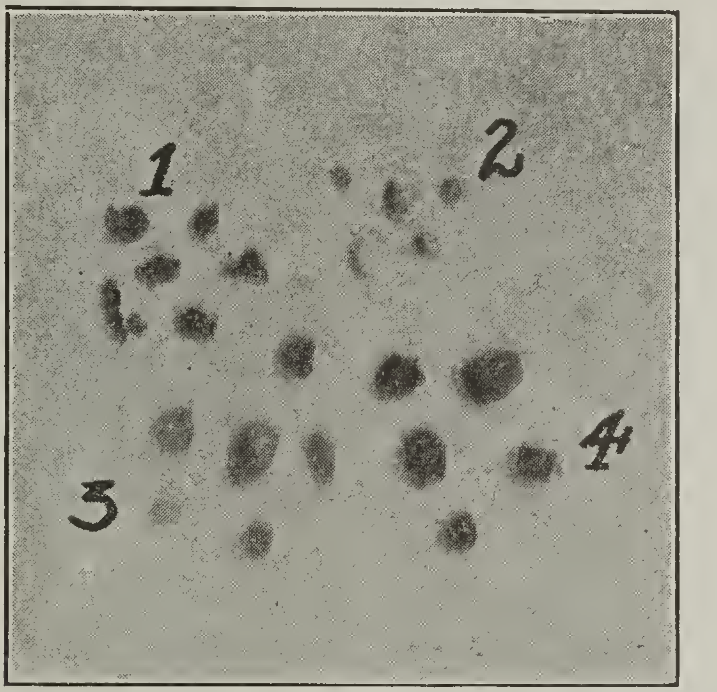

Fig. 8. A Photograph Showing the Foreign and Other Hard Bodies of the Excreta Which Resisted Grinding or Maceration and Was Removed by Aid of the Sieve

1. Grit or quartz.
2. Pieces of cinders.
3. Masses of hard uric acid crystals.
4. Masses of undigested middlings.

Chemical Analyses of Excreta

The methods used in determining the moisture, ash, ether extract, crude fiber, nitrogen-free-extract, and total nitrogen are the same as those used in the feed analyses.

Determination of Free Ammonia

Place 0.7 gm. of the excrement in a Kjeldahl flask with 200 cc. of water and 5 gms. of magnesium oxide free of carbon dioxide. Then connect the flask with a condesner and distill 100 cc. of the liquid into standard acid, titrate and calculate the ammonia present.

Determination of Uric Acid

Take ten grams of thoroughly mixed ground feces, place in a 250 cc. beaker and treat with 100 cc. of 95% alcohol. Filter through paper and wash twice with 30 cc. of alcohol, then wash three times with ether and allow to dry. This removes the greater part of the coloring and fatty matter. The residue when dry is returned to the beaker. One hundred cc. of 5% solution of HCl is added and set in a refrigerator over night.

The following morning the material is filtered through paper and washed twice with cold water, returned to the beaker with 100 cc. of water and enough piperidine added to dissolve the uric acid. A few drops of phenolphthalein were added to the solution in order to determine the amount of alkali necessary to dissolve all the uric acid, also the disappearance of the white particles from the bottom of the beaker indicates complete solution. It usually requires from one-half to three-fourths of an hour heating on the water bath with frequent stirring to effect complete solution. After the white particles all diappear and the solution remains alkaline it is passed through a coarse linen filter into a five hundred cc. graduated flask, washed thoroughly with hot water, squeezing out, filtered two or three times to facilitate washing, cooled and made up to the mark and thoroughly mixed. The solution is then allowed to settle until the fine particles which passed through the cloth settle out, leaving a clear solution which can be drawn off. The solution is allowed to settle over night so that it will be clear in the morning.

Fifty cc. portions, representing 2 gms. of the feces, are taken, made acid with HCl, evaporated on a water bath to 25 cc. and set in a refrigerator over night, or better still for twenty-four hours. Then filter through a 597 S. & S. filter paper on a Buckner's funnel 50 mm. in diameter, under pressure, wash three times with cold water, then with absolute alcohol, and lastly two or three times with ether to remove any trace of remaining fat. The uric acid is thus collected on a very small filter paper which is transferred to a beaker, boiled with 35 cc. of distilled water and titrated with N—10 piperidine solution. It is very necessary to complete the end reaction with the solution as near the boiling point as possible, or concordant results cannot be attained. All determinations are run in triplicates. The first titration is made roughly to find out the approximate end point. In titration, as the end point is being reached, the beaker is again put over the flame and brought to boiling point, then titrated to completion.

To test the accuracy of the method pure uric acid is added to feces that contain none. The average determination in our case gave 98.2 percent of acid recovered.

The piperidine solution is standardized with uric acid which is purified by twice recrystalizing and found practically pure.

THE PREPARATION OF PIPERIDINE FROM PYRIDINE AND SODIUM

Action of metallic sodium in solution of pyridine in absolute alcohol.

Absolute alcohol was made from 95% alcohol by dehydrating it with calcium oxide and redistillation at 80 degrees to 90 degrees C.

One hundred cc. pyridine was dissolved in 1800 cc. of absolute alcohol and 156 gms. of metallic sodium gradually added till all was dissolved. Sodium ethylate was removed by filtration three times during the adding of the sodium.

Excess alcohol was distilled. Portion of distillate caught at 80 degrees to 82 degrees C. and was saved for further use.

The portion of the distillate caught at 105 degrees to 110 degrees C. was saved as piperidine.

The distillate caught between 105 degrees to 110 degrees C. was very strongly alkaline to phenolphtalien and caused uric acid to dissolve, decolonizing the phenolphthalein color.

DISCUSSION

The constituents of mammalian urine are classed as organic and inorganic. The chief organic constituents are nitrogenuous end products—urea, uric acid, hippuric acid, creatin, and creatinin; aromatic compounds—benzoic acid, etherial sulphates of phenol, cresol, and small amounts of animo acids, allantoin, purin bases and coloring matter and mucous[8]; salts consisting of sulphates, phosphates and chlorides of sodium potassium, calcium, and magnesium.

The total nitrogen of the urine consists of urea nitrogen, uric acid nitrogen, ammonia nitrogen and creatinin nitrogen.

Creatinin is hydrolized creatin. It has been generally considered that creatinin of the urine arises from the creatin of the muscles. In alkaline urine creatin appears in greater quantity while the reverse is the case with strongly acid urine.[9]

Urea originates in the body partly from retrograde tissue metamorphosis, including the blood, and partly from splitting up of unassimilated nitrogenuous principles of the food.

Uric acid in birds is formed in the liver from ammonia compounds and lactic acid. It is formed from the splitting of nucleoproteins[10]. These nuclein bodies are compounds of protein with nucleic acid, the latter constituent splitting up into thymic acid and derivatives of purin among which is uric acid, xanthin, hypozanthin, etc.

The ammonia salts present in urine are an index to the neutralization of acids in the body. The acid substances are produced as the result of metabolism. When they are in excess there is an increase in the ammonia of the urine, the formation of ammonia in the muscles being the natural protection of the body against acid poisoning.

Hippuric acid is a product of herbivora.

Xanthin is contained in human urine.

Brown says that the nitrogenuous substances of birds' urine, aside from ammonia urate, are present in exceedingly small quantities. In fact it must be borne in mind that the creatinin, hippuric acid, purin bases, etc., are present in mammalian urine in relatively minute quantities, and this is conspicuously brought out when the amount of such extractives are·calculated per kilo weight. Hence the urine of animals of so low a body weight as poultry would contain in all probabilities but minimal amounts of such exactives if present at all. In view of these considerations very large quantities of excrement would be required to demonstrate the presence of such bodies.

Meissner, in 1861, found in hens fed on meat the urine yielded creatin but no creatinin. Only minute amounts were eliminated by the grain-fed birds, and in many cases its presence could not be satisfactorily demonstrated. Liver-fed hens produced less than those fed on meat When creatin was given hypodermically it was practically all recovered as such in the excrement. He claims that two to four percent of the urinary nitrogen is urea. He did not find hippuric acid in the urine of hens.

Milroy says that urine of hens may contain in very small quantities purin bases.

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[8] Smith, Col. F., Veterinary Physiology, p. 292.

[9] Purdy, Practical Urine Analysis, p 36.

[10] Webster, Diagnostic Methods, p 218.

Owing to the smallness and shortness of the large intestine and the rapidity of the digestive processes of the hen and the quick passing out of the fecal matter, there would be expected to be a relatively small amount of putrefaction and hence small amounts of indol, skatol, and bodies of that type.

Cristiani detected etenol sulphuric acid in a hen's urine after a meat diet and found that the injection of indol leads to the excretion in the form of indoxylsulphuric acid.

THE ASH INTAKE AND ASH OUTGO AND ITS EFFECT UPON THE QUESTION OF COEFFICIENTS OF FEEDING STUFFS WITH POULTRY

During the time when the first series of digestion coefficients were being summarized it was observed that a rather large percentage of ash was found in the excrement. As shown in an earlier part of this paper grit is continually being voided.

An unsuccessful effort was made to separate the grit from the excreta, both sieve and forces methods being used. It was decided to then carry on a series of experiments extending over a period of twelve months to determine the fate of grit in the body of the fowl.

The analytical method consisted of the following proceedings:

Six hens were brought off range from the poultry plant of the Station and placed in the feeding coops used for the digestion coefficient feeding experiments. The hens were numbered one to six, inclusive.

The following mash and grain rations were fed:

MASH

Wheat middlings	60 pounds
Ground oats	10 pounds
Corn meal	15 pounds
Meat scrap	15 pounds

GRAIN

Corn	50 pounds
Oats	50 pounds

The mash had an ash content of 4.1 percent. The grain mixture had an ash content of 2.1 percent. On the thirteenth day of this experiment a new batch of mash was secured having the same feeds, but having an ash content of 4.7 percent.

The hens were fed mash in the morning and grain in the afternoon. The excreta was collected every twenty-four hours, dried at a temperature of 106 degrees C. in an electric oven and weighed. The excreta was then finely pulverized in an iron mortar. From this finely pulverized excrement 0.5 gm. sample was taken and an ash determination made according to the Official Method of Analysis, Bulletin No. 107, revised, Bureau of Chemistry, U. S. Department of Agriculture. The ash determinations were always made in duplicate and were required to check within 0.3 of one percent.

There will be given here only the final summary table showing the percent feed digested during the normal four day digestion period and the percent digested when the excess ash is taken into consideration with hens 1, 3, 5, and 6. The difference being the final correction factor to be applied to the digestion coefficients tables for poultry.

TABLE I

Summary Table Showing Ash Intake and Ash Outgo of Five Hens

Days	Total Amount Feed	Total Amount Excrement*	Percent Digested	Total Amount Excess Ash	Percent Digested	Difference in Percent
5th	226	85,9	61,97	4,35	64,06	2,09
6th	230	102,0	55,23	6,86	58,31	3,08
7th	248	105,8	56,34	6,71	59,28	2,94
8th	256	99,5	60,95	6,07	63,39	2,44
Total and averages	960	393.2	58.62	24.17	61.26	2.64**

*In grains.

**Final correction factor.

From this table it is seen that the correction factor under the conditions under which these experiments were run is 2.64 percent. That is the average ash thrown off in the form of grit between the fourth and eigth days amounted to 2.64 percent.

Graph 3 from hen 3 is given as it is an average result and will illustrate the curve of ash intake and outgo from the first day off of grit to the one hundredth day and then what happens when grit is then given and then to the 114th day.

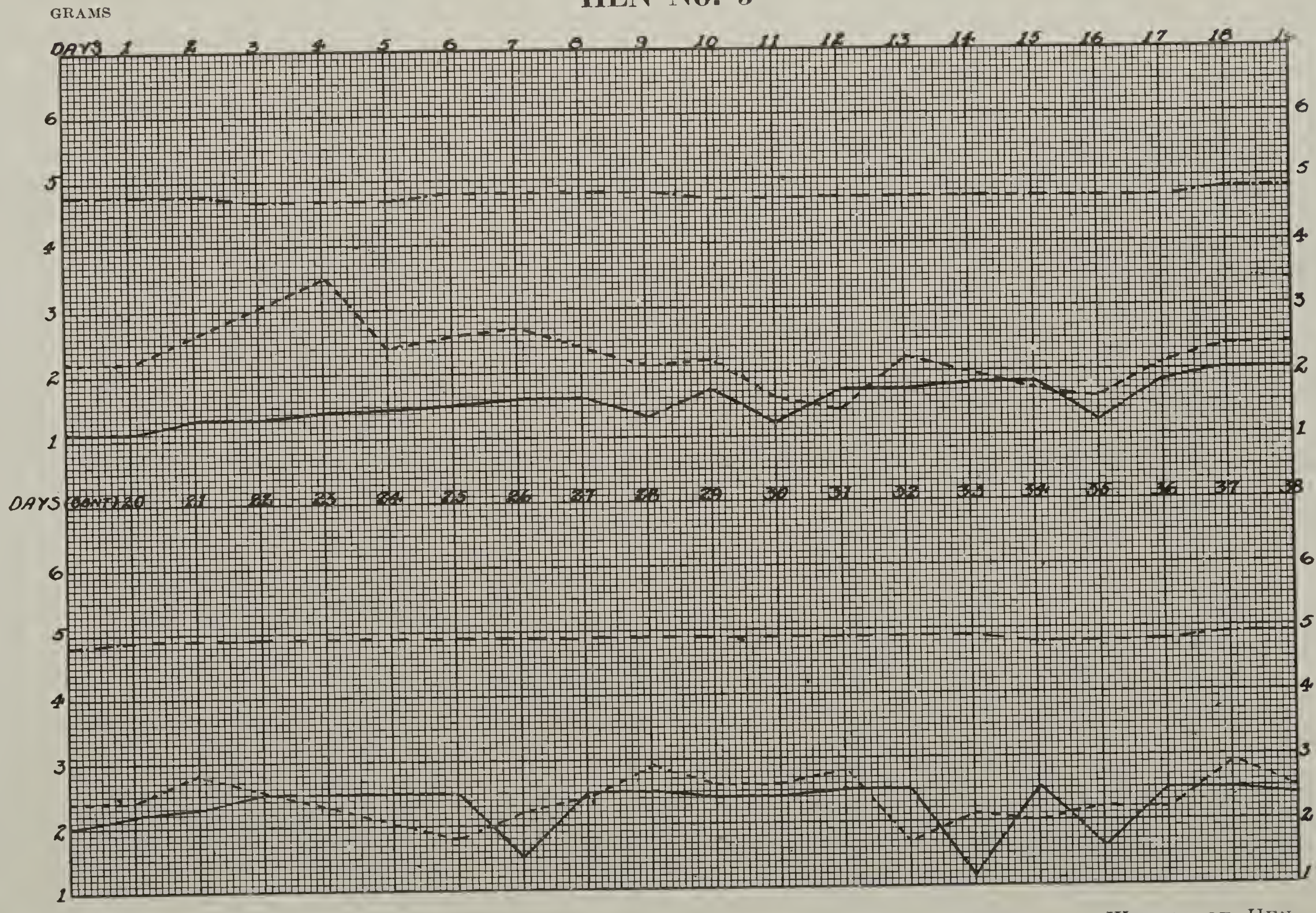
Hen No. 3 represented in the graph consumed 1.1 grams of ash in the feed the first day and gave off 2.2 grams of ash. The ash outgo increased until the fourth day, when it reached 3.5 gm. From the fourth to the twelfth day it consistently decreased until on the twelfth day the ash outgo was three-tenths of a gram less than the ash intake. From the twelfth to the one-hundred-second day the intake and outgo lines are constantly crossing and recrossing each other. On the one hundredth day a cup of grit was placed before the bird. There was an increase in ash outgo from the one hundred and first to the one hundred and third day, there was then a slight drop till the one hundred and sixth day and a continuous increase from the one hundred and sixth to the one hundred and fourteenth day when the experiment was discontinued.

A summary study of all the tests show that the ash outgo for the first twelve days is always greater than the ash intake, thus indicating that the residual amount of ash is reached on or about the twelfth day. The graphs also show that as long as the bird is deprived of grit that the intake and outgo lines continuously cross each other, but upon giving the bird free access to grit on the second day there is a considerable increase in the ash outgo over that of the intake in the feed.

This indicates that when the bird has free access to grit on range that she is continuously taking in new and fresh grit.

Thus in Table I is given a final summary of the amount of feed consumed during each day of the normal four day digestion period, total amount of excrement voided and the percent of feed digested. The total amount of excess ash voided each day, the percent digested when the excess ash is taken into consideration and the difference is the correction factor. During the four day normal digestion period the five hens consumed 960 gms. feed. They gave off 393.2 gms. excrement, showing 58.62 percent digested. They gave off 24.17 gms. more ash than they took in in the feed. When the excess ash was deducted from the excrement and the percent feed digested it shows that 61.26 percent is digested. A difference of 2.64 percent. This is the final correction factor to be applied to the digestive coefficient tables.

HEN No. 3

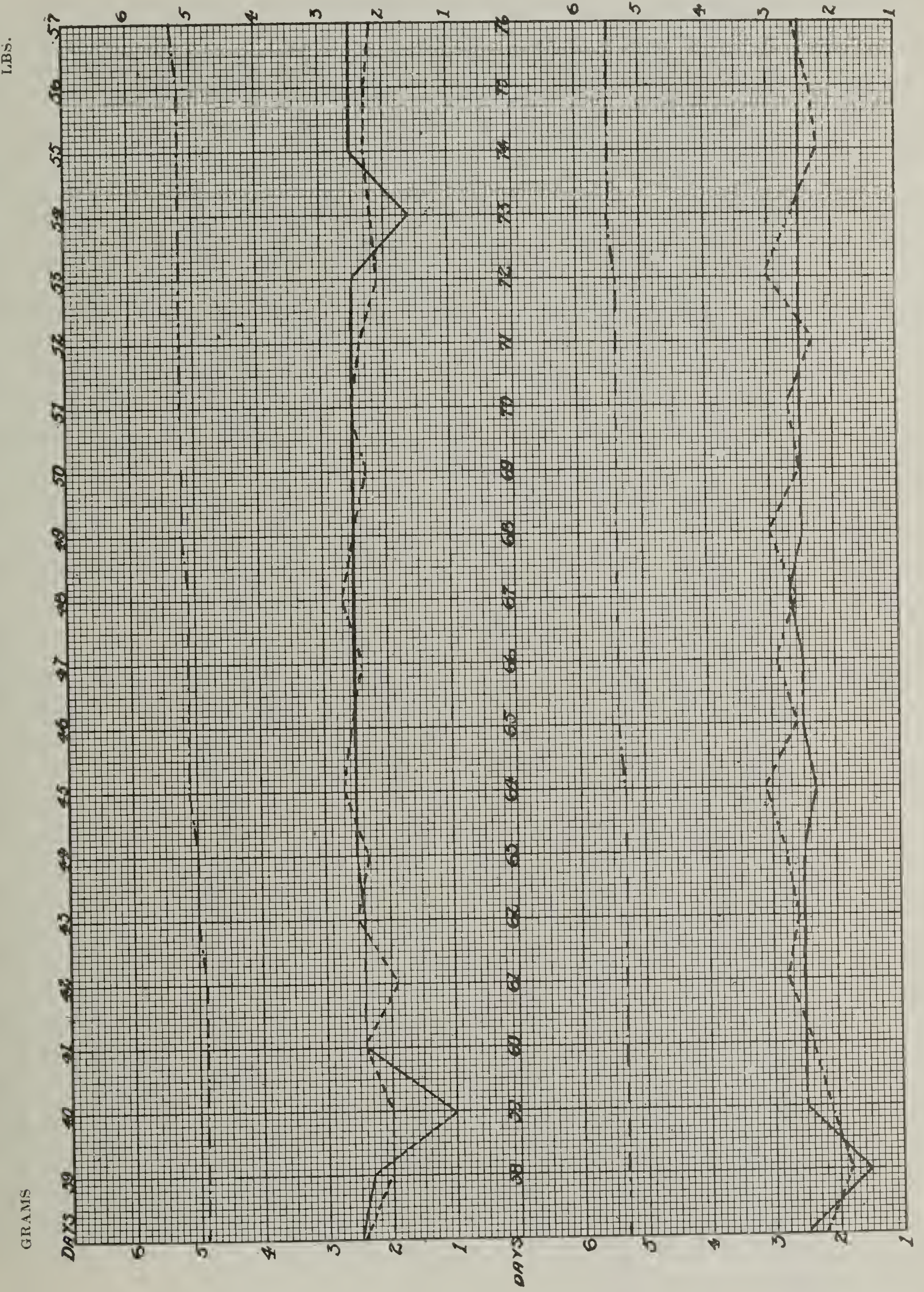


Graph of Hen No. 3 Showing Ash Intake With Feed and Ash Outgo in Excreta and Weight of Hen.

— – — weight of hen, lbs. ———— ash intake, grams. — — — — ash outgo, grams

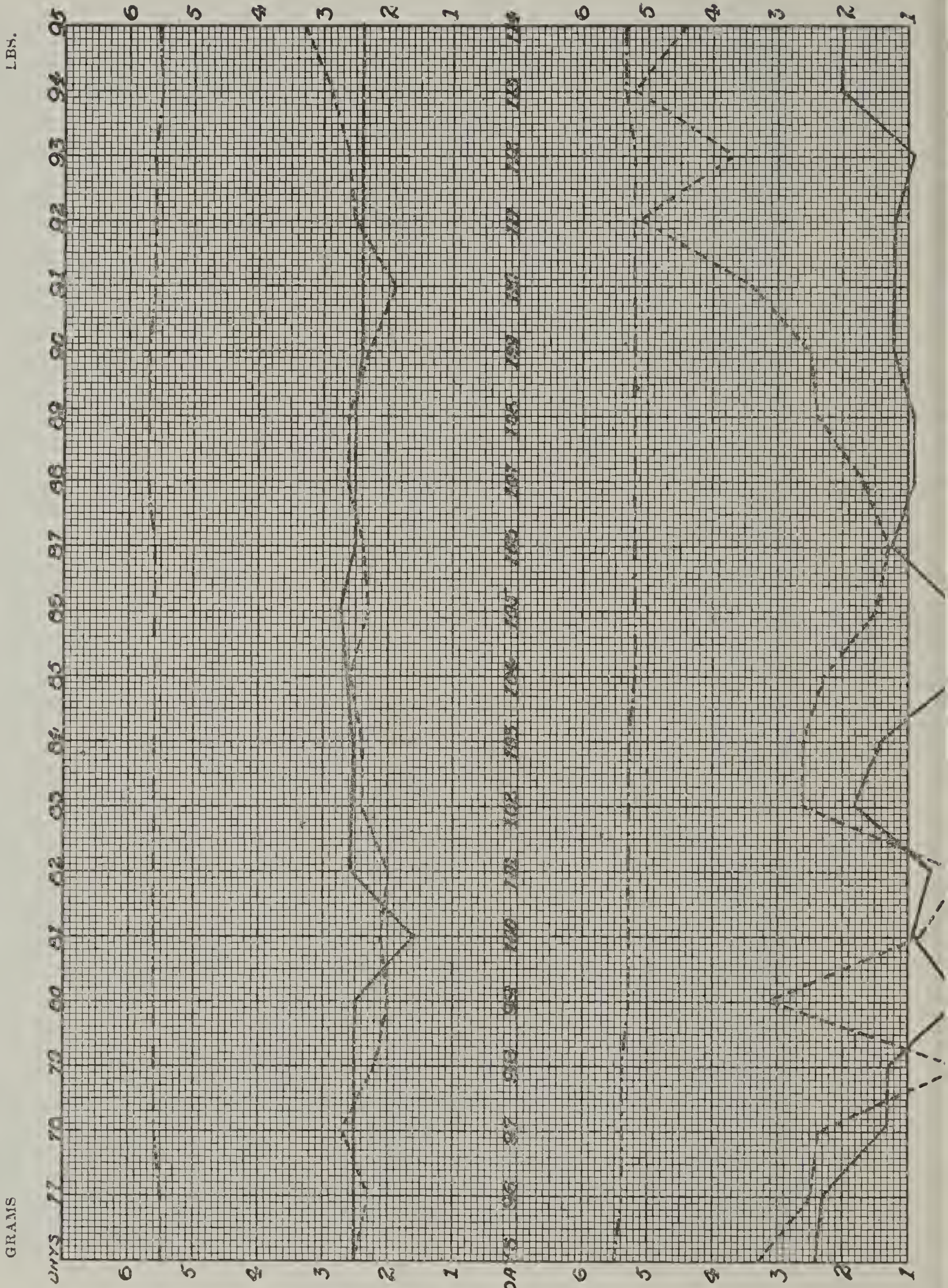
HEN No. 3

(continued)



HEN No. 3

(CONTINUED)



THE FATE OF GRIT IN THE FOWL

Since the fowl has no teeth with which to grind its feed it is common knowledge that the muscular walls of the gizzard contracting upon its contents, namely, food and grit, reduces the food to fineness. The problem is to make an effort to determine how long such grit is useful in the gizzard and how often it must be replenished. Does a hen constantly consume more than she really requires, and if so, is there a tendency to a residual amount constantly kept in the gizzard?

Fowls of the American breeds were used in the experiment. Only hens were tested and birds two to three years old were used in the test. The hen could not be expected to lay under the conditions in which they were required to be kept.

Thirty-six hens, all Barred Plymouth Rocks, were kept in coops eighteen inches square The coops were provided with one inch mesh wire netting bottoms so that the excreta would immediately pass through to a second floor as soon as voided. This prevented a possible reconsumption of any grit passed in the excreta. Hens were killed at different times and the gizzard content examined for the grit which still remained.

Analyses of the intake and outgo of the feed, and the weight of the birds, were taken as an indication as to whether the grit content of the gizzard was sufficient for normal physiological process of that organ.

The feed for 365 days, the duration of the test, consisted of regular scratch feed and dry mash used on the College and Station poultry plant.

Since the detail tables are quite voluminous it is thought best to give only the summary table and to show the entire series of tubes showing the amount of grit left in the gizzard at the end of the various periods. The following tabulation will serve the purpose of a comparative study of the amount of grit.

TABLE II

The Amount of Grit Remaining in the Gizzards of Hens at the End of Each Period

Number Days Without Grit	Weight of Hens at Start (lbs.)	Weight of Hen When Killed	Weight of Grit in Grams	Remarks
14	5.2	5.4	9.5012	Killed
14	6.4	6.3	13.1136	Killed
21	4.5	4.8	8.3126	Killed
21	5.3	5.8	16.9326	Killed
28	4.6	4.9	11.8763	Killed
28	7.0	7.3	22.6531	Killed
36	4.8	5.7	16.4389	Killed
36	2.7	4.0	5.0378	Killed
42	7.0	4.2	8.4531	Died of Sarcoma
93	3.6	3.2	11.6341	Killed
120	3.8	6.2	4.9643	Killed
124	5.8	6.1	5.6321	Killed
143	5.6	4.9	4.7532	Died of Mites
144	7.0	5.8	9.5923	Killed
150	5.7	5.7	6.5120	Died of Mites
153	7.1	5.6	4.5644	Died of Mites
154	6.5	5.5	5.9633	Killed
156	6.8	5.8	14.0326	Died of Mites
170	2	2	9.8670*	Died
248	6.2	6.1	2.5200	Killed
270	6.3	6.6	5.0000	Killed
300	5.7	5.9	3.9525	Killed
330	5.1	6.1	1.9530	Killed
365	6.2	5.2	2.5610	Killed
375	6.7	7.1	5.8915	Killed

*This cockerel was sent to the laboratory when about two pounds in weight. It remained in the coop till it died on the 170th day after coming to the hospital. It was affected with partial paralysis from which it never entirely recovered.

DISCUSSION

The feed records show that the appetite kept up fairly well for birds not having exercise. This is of interest in view of the fact that it is a check on the hens which were used in the digestive coefficient work where with single feeds the birds soon went off feed. In these cases the fowls received a variety of feed.

From these preliminary tests it is seen that a bird may go 365 days without being fed grit, and have plenty of grit remaining in its gizzard with which to grind its feed. The grit that had remained in the gizzard 365 days appeared just as sharp as that found in the gizzard at the commencement of the tests. In fact we do not believe that the grinding process in the gizzard of the fowl is one of sharp cutting processes, but rather the following process. The food soaks more or less in the crop depending on the length of time it remains there. The food passes from the crop through the second portion of the esophagus to the proventriculus where it soaks in a strong acid secretion. From the proventriculus the food passes into the gizzard where the muscles of the walls of the gizzard contract, squeezing the soaked grain among the grit and by a squeezing rotary motion the food is reduced to fineness and is more like the action of a ball mill. Birds hold their weight and remain perfectly healthy on either sharp or dull grit.

There is a tendency for a fowl to eat more grit than is essential for grinding the food. In another series of experiments we have shown that the amount of mineral given off for the first twelve days was much more than that taken in. Further experiments showed that this was due to the grit passed off from the gizzard. While there is a tendency to pass off the excess grit and to keep a residual amount, yet the amount retained varies greatly with different individuals.

One hen at the end of 365 days had 5.89 grams of grit, as much as one of the hens that was killed for examination on the thirty-sixth day. One hen that died on the one hundred and fifty-sixth day had 14.03 gms., or more than that contained by the gizzards of the hens killed on the fourteenth day and twenty-first day. As shown by the health of the birds, a fowl may go longer than a year without replenishing her grit.

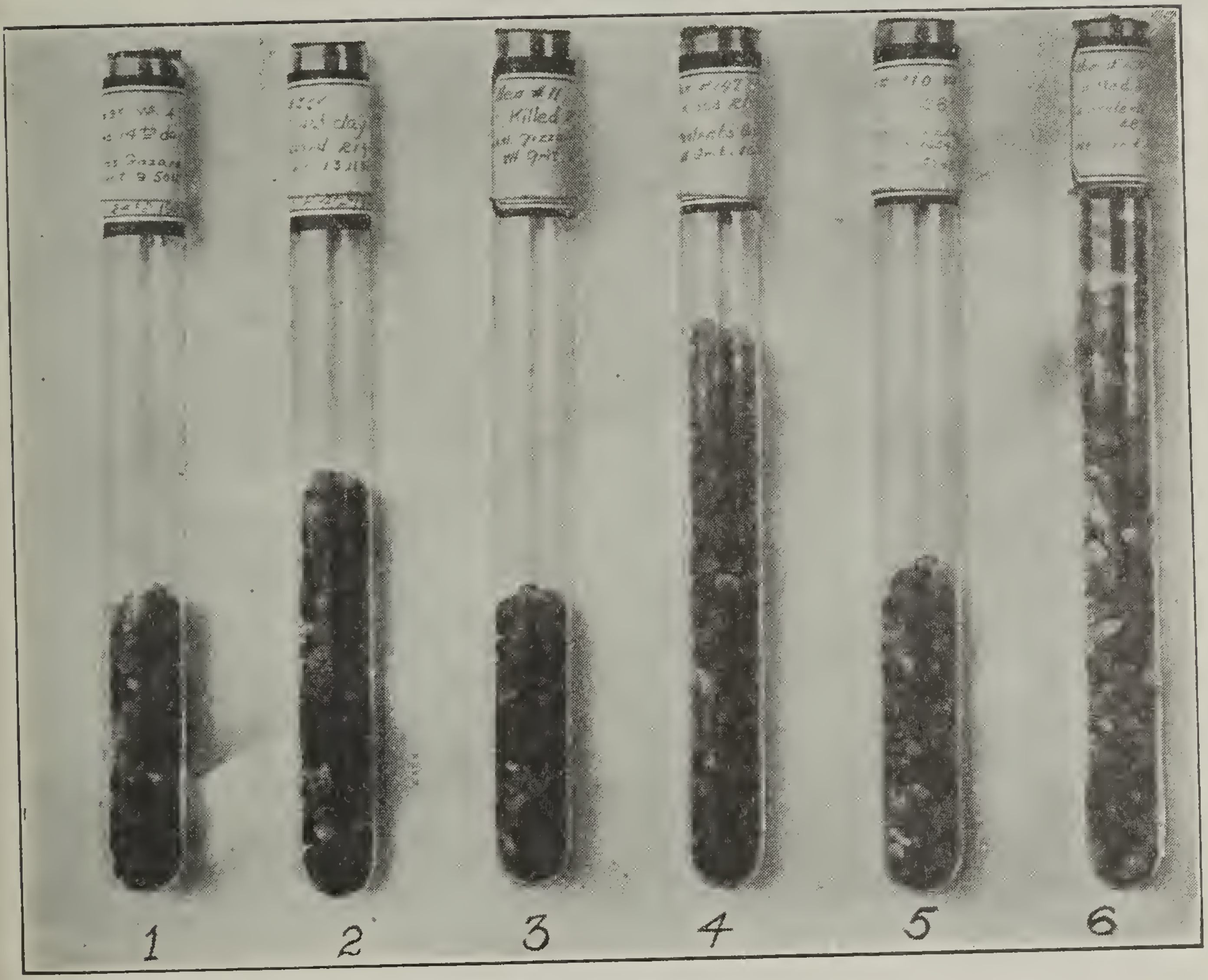


Fig. 9. Amount of Grit Remaining in the Gizzards of Hens at the End of Each Period.

Hen No. 1, killed 14th day. Wt. gizzard, 13.00 gms. Wt. grit, 9.50 gms.
Hen No. 2, killed 14th day. Wt. gizzard, 21.00 gms. Wt. grit, 13.11 gms.
Hen No. 3, killed 21st day. Wt. gizzard, 12.01 gms. Wt. grit, 8.31 gms.
Hen No. 4, killed 21st day. Wt. gizzard, 19.03 gms. Wt. grit, 16.93 gms.
Hen No. 5, killed 28th day. Wt. gizzard, 13.46 gms. Wt. grit, 11.87 gms.
Hen No. 6, killed 28th day. Wt. gizzard, 28.19 gms. Wt. grit, 22.65 gms.

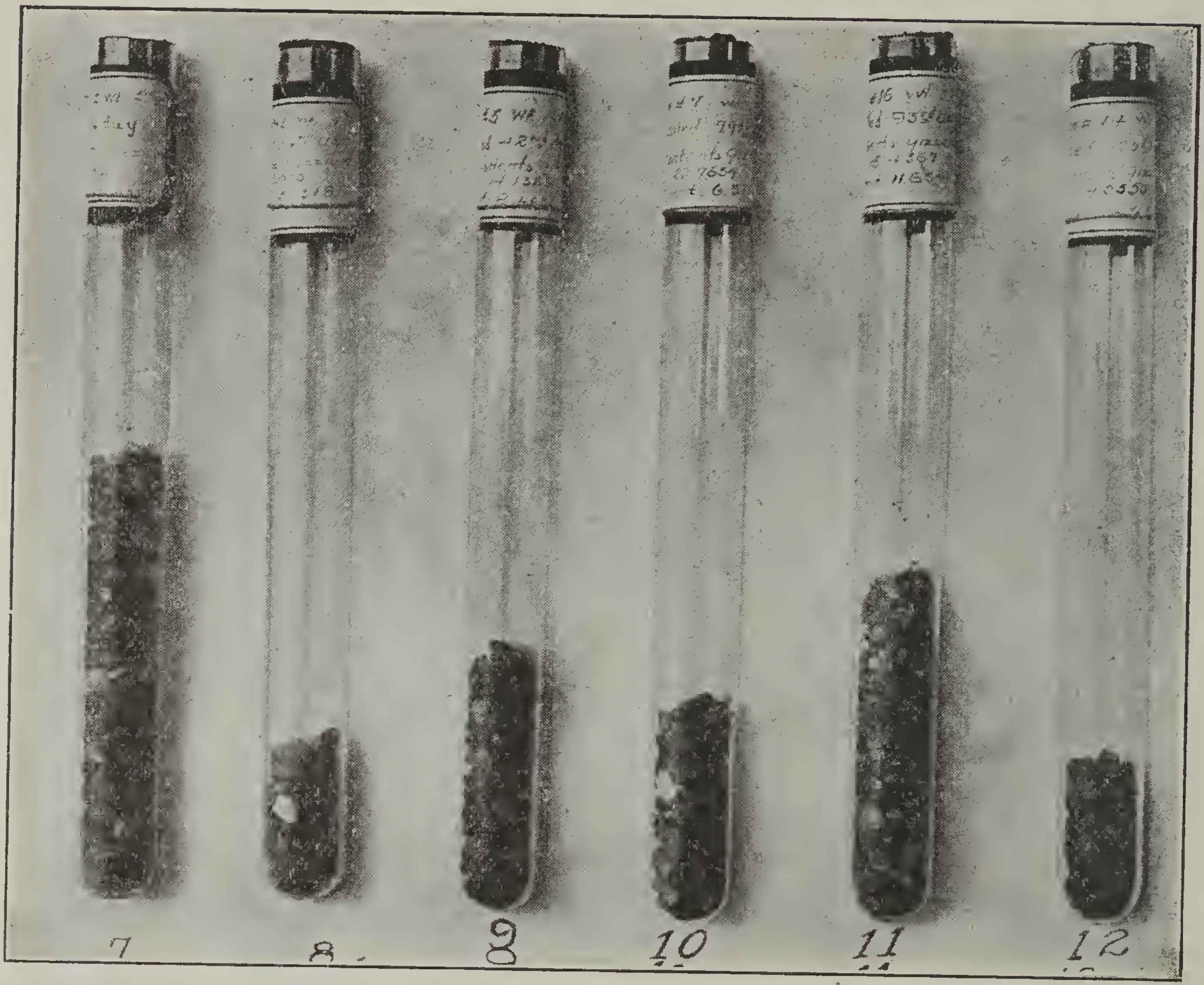


FIG. 10. AMOUNT OF GRIT REMAINING IN THE GIZZARDS OF HENS AT THE END OF EACH PERIOD.

Hen No. 7,	killed 36th day.	Wt. gizzard, 19.00 gms.	Wt. grit, 16.43 gms.
Hen No. 8,	killed 36th day.	Wt. gizzard, 7.10 gms.	Wt. grit, 5.03 gms.
Hen No. 9,	killed 42d day.	Wt. gizzard, 14.13 gms.	Wt. grit, 8.46 gms.
Hen No. 10,	killed 79th day.	Wt. gizzard, 20.76 gms.	Wt. grit, 6.37 gms.
Hen No. 11,	killed 93d day.	Wt. gizzard, 15.43 gms.	Wt. grit, 11.63 gms.
Hen No. 12,	killed 120th day.	Wt. gizzard, 9.63 gms.	Wt. grit, 4.96 gms.

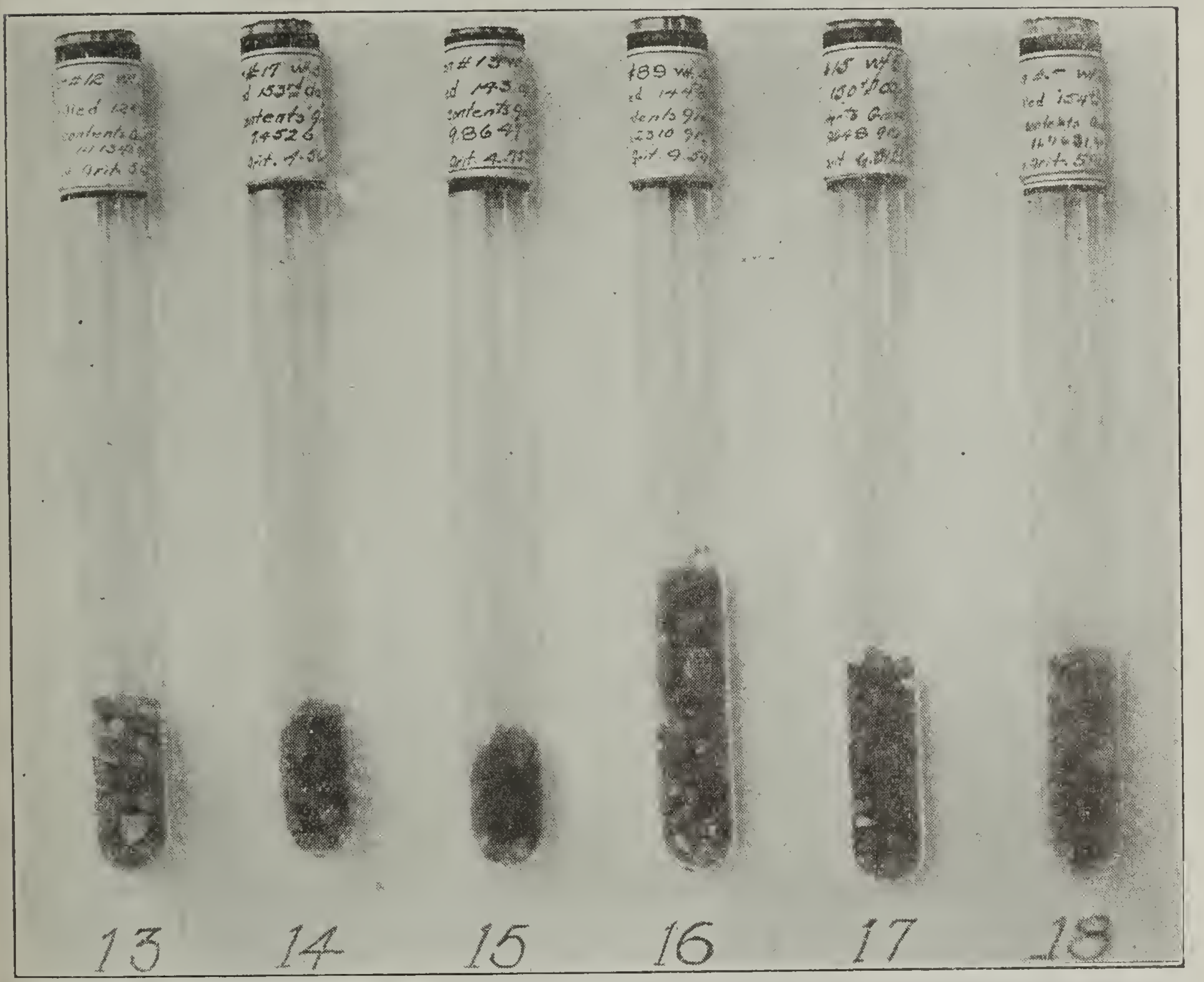


FIG. 11. AMOUNT OF GRIT REMAINING IN THE GIZZARDS OF HENS AT THE END OF EACH PERIOD.

Hen No. 13, killed 124th day.	Wt. gizzard, 14.13 gms.	Wt. grit,	5.63 gms.
Hen No. 14, killed 143d day.	Wt. gizzard, 9.86 gms.	Wt. grit,	4.75 gms.
Hen No. 15, killed 143d day.	Wt. gizzard, 9.86 gms.	Wt. grit,	4.75 gms.
Hen No. 16, killed 144th day.	Wt. gizzard, 13.23 gms.	Wt. grit,	9.59 gms.
Hen No. 17, killed 150th day.	Wt. gizzard, 11.96 gms.	Wt. grit,	6.51 gms.
Hen No. 18, killed 154th day.	Wt. gizzard, 11.76 gms.	Wt. grit,	5.96 gms.

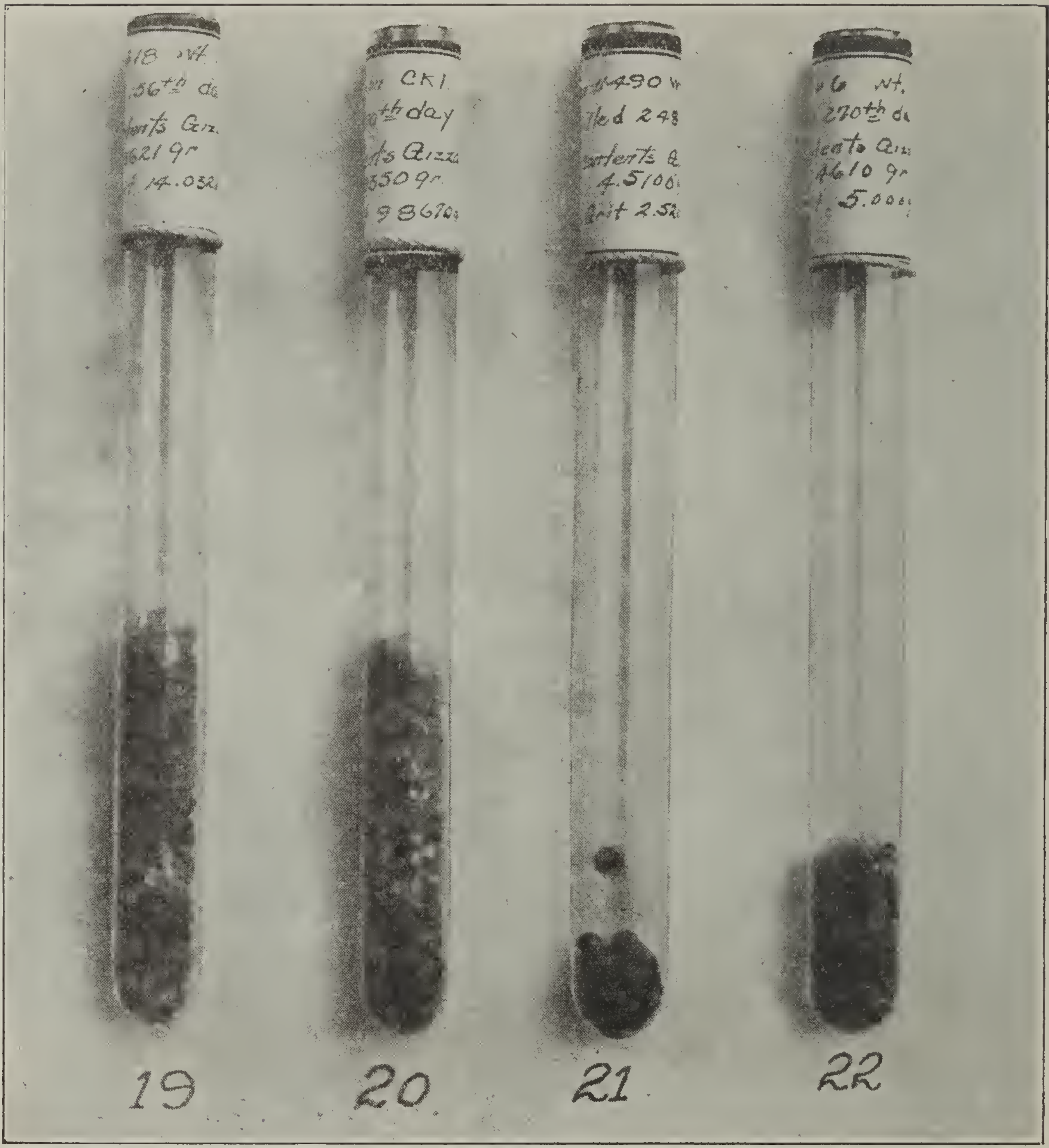


FIG. 12. AMOUNT OF GRIT REMAINING IN THE GIZZARDS OF HENS AT THE END OF EACH PERIOD.

Hen No. 19, killed 156th day. Wt. gizzard, 17.36 gms. Wt. grit, 14.05 gms.
Hen No. 20, killed 170th day. Wt. gizzard, 14.93 gms. Wt. grit, 9.86 gms.
Hen No. 21, killed 248th day. Wt. gizzard, 4.51 gms. Wt. grit, 2.52 gms.
Hen No. 22, killed 270th day. Wt. gizzard, 10.46 gms. Wt. grit, 5.00 gms.

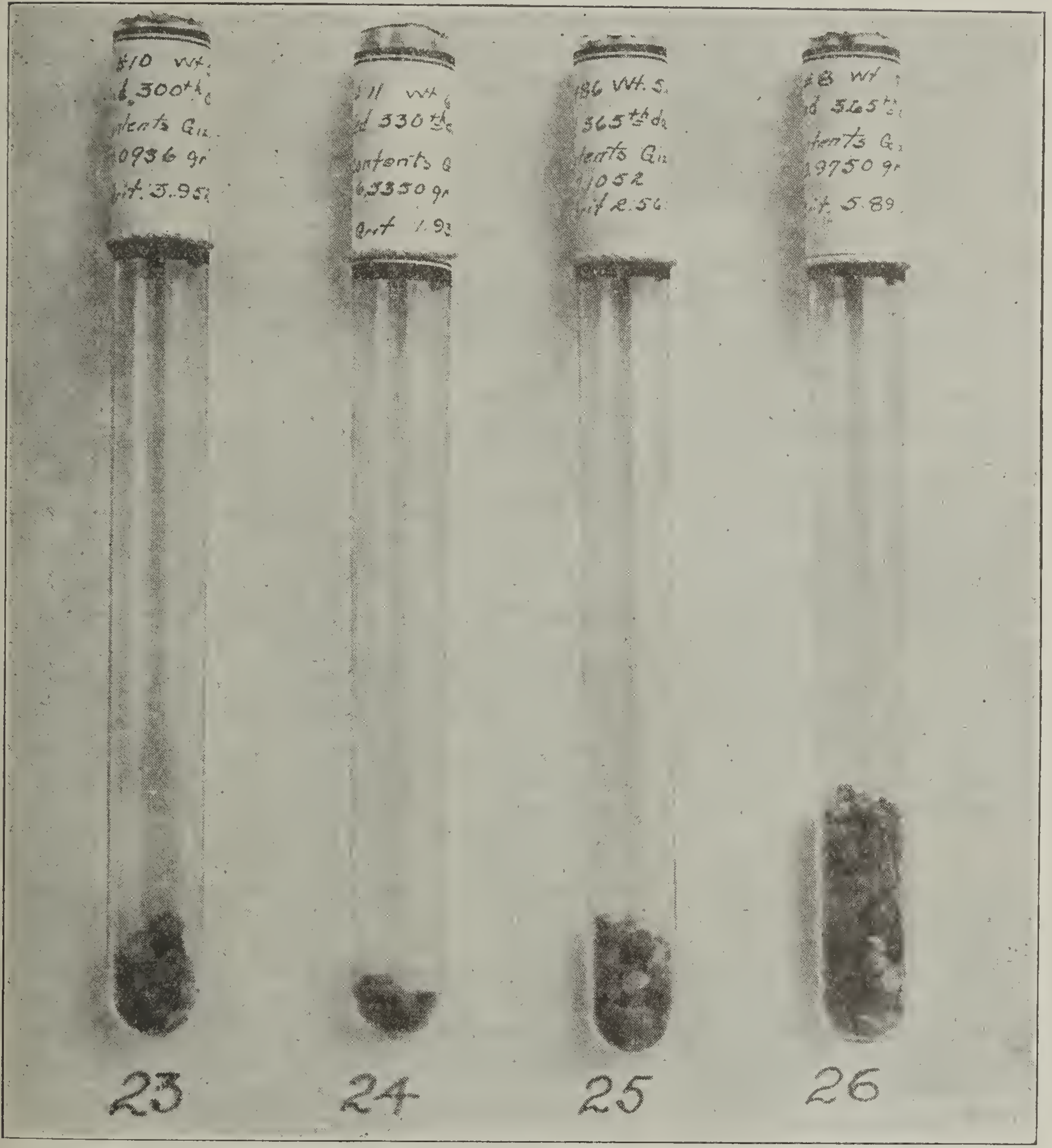


Fig. 13. Amount of Grit Remaining in the Gizzards of Hens at the End of Each Period.

Hen No. 23, killed 300th day. Wt. gizzard, 10.00 gms. Wt. grit, 3.95 gms.
Hen No. 24, killed 330th day. Wt. gizzard, 6.53 gms. Wt. grit, 1.93 gms.
Hen No. 25, killed 365th day. Wt. gizzard, 7.10 gms. Wt. grit, 2.56 gms.
Hen No. 26, killed 365th day. Wt. gizzard, 9.97 gms. Wt. grit, 5.89 gms.

TABLE III

PALATABILITY OF SINGLE FEEDS AND COMBINATIONS

Kind of Feed	Letter No.	Breed Used	No. Started	No. Finished	Percent Finished
Wheat middlings	A	White P. Rocks Buff Rocks Buff Orpingtons	9	9	100
Corn	B	Buff Rock	12	5	41
Wheat	C	White Rock, Buff Rocks	24	6	25
Oats	D	Buff Orpington	12	6	50
Corn meal (bolted)	E	Buff Plymouth Rock	12	5	41
Beef scrap and bone meal, 1-3 Corn meal—2-3	F	White Plymouth Rock	6	2	33
Tankage—20% Cornmeal—80%	G	White Plymouth Rock Buff Orpingtons	6	2	33
Blood meal—20%, Corn meal—80%	H	Buff Plymouth Rock	6	4	66
Buttermilk (dried),—20%, Cornmeal—80%	I	Partridge Plymouth Rocks	6	5	83
Soybean meal (fat extracted) —1-3, Corn meal—2-3	J	Barred Plymouth Rocks	6	5	83
Peanut meal—1-3, Corn meal —2-3	K	Barred Plymouth Rocks	6	5	83
Fish meal-20%, Corn meal-80%	M	S. C. Rhode Island Reds	6	5	83
Cottonseed meal —1-3, Corn meal—2-3	N	Golden Wyandotte	6	4	66
Corn meal (unbolted)	O	Barred Plymouth Rocks	6	3	50
Wheat middlings	P	Buff Orpingtons	12	6	50
Rye	Q	Silver Pencil Wyandotte Barred Rocks	6	0	00
Rye—1-4, Corn—3-4	Q	Silver Penciled Wyandottes	6	3	50
Rye—50%, Corn meal—50%	Q	Silver Penciled Wyandottes	6	0	00
Hulled Oats	R	R. I. Reds	6	5	83
Buckwheat—1-4, Corn meal—3-4	S	Barred Plymouth Rocks	6	6	100
Rice—1-4, Corn meal—3-4	T	Rhode Island Reds	6	5	83
Kafir corn—1-4, Corn meal—3-4	U	Silver Penciled Wyandottes	6	5	83
Barley—1-4, Corn meal—3-4	V	Buff Orpingtons	3	2	66
Wheat Middlings—1-4, Corn meal—3-4	W	Barred Plymouth Rocks	4	3	75
Total	Single Feed		105	48	45.7
	Combination		79	53	67.4

BEHAVIOR OF BIRDS ON SINGLE FEEDS

The palatability of single feeds as shown by our feeding tests is of considerable interest. In selecting our fowls we chose mature hens of lymphatic temperament. Such hens did not worry in confinement. It appears that the appetite of fowls does not always hold up well on a single feed as it does on a variety.

The feeding tests were run in series of six hens each, one hen in each cage as previously described. The feeding tests extended over a period of eight days. The birds were given a definite number of grams of the feed at 7:30 a. m. and 4:30 p. m.

The breeds of fowls used in these tests were Buff and White Plymouth Rocks and Buff Orpingtons. In the first series one hundred percent finished and the second series fifty percent finished the test. It is also of interest to note that in the test with wheat middlings and corn meal, of the four that started, three, or 75 percent, finished the test. Wheat middling has a tendency to cause a looseness of the bowel on about the fourth day. This seemingly has a tendency to cause the bowel to unload.

Of the twelve Buff Plymouth Rock hens that started on corn, but five, or 41 percent, finished. This put wheat middlings in these tests ahead of corn in palatability.

Of the twenty-four White and Buff Plymouth Rock hens starting on wheat but six, or 25 percent, finished which was the lowest percent of any of the tests to go through.

Of the twelve Buff Orpington hens starting on oats, six, or fifty percent, finished, putting oats slightly ahead of either corn or wheat.

Of the twelve Buff Plymouth Rocks starting on bolted corn meal, only five, or 41 percent, finished, putting it in the class of corn, while of the six Buff Plymouth Rock hens starting on unbolted corn meal, three, or fifty percent, finished.

Of the six Silver Penciled Wyandottes starting on rye and corn meal none finished, but of six Partridge Plymouth Rocks starting on rye and corn meal in a third test, three, or fifty percent finished. Thus of eighteen hens starting on rye in full or as part feed only three or sixteen percent finished. The hens on 100 percent rye developed mild diarrhoea on the second day and severe diarrhoea by the fifth day. The hens on fifty percent rye and fifty percent corn meal developed mild diarrhoea by the fourth day but the diarrhoea did not become severe. The hens on 25 percent rye and 75 percent corn meal did not develop diarrhoea.

Of the three Buff Orpington hens starting on one-fourth part of barley and three-fourths part corn meal , two, or 66 percent, finished.

Of the six Silver Penciled Wyandottes starting on Kafir corn one-fourth part, and corn meal three-fourths part, five, or 83 percent, finished.

Of the six S. C. Rhode Island Red hens starting on one-fourth part rice and three-fourths part corn meal, five, or 83 percent, finished, thus putting rice high as a desirable feed from a tolerance standpoint.

Slightly better than rice was buckwheat. Six Barred Plymouth Rock hens were placed on buckwheat one-fourth part and corn meal three-fourths part with 100 percent finishing the test.

Of six S. C. Rhode Island Red hens starting on hulled oats, five, or 83 percent finished, placing hulled oats ahead of oats.

Of six Golden Wyandotte hens starting on cotton seed meal one-third and corn meal two-thirds, four, or 66 percent, finished the test.

Of six S. C. Rhode Island Reds starting on 20 percent fish meal and 80 percent corn meal, five, or 83 percent, finished, placing fish meal high as a feed from a tolerance standpoint.

Of six Barred Plymouth Rock hens starting on peanut meal one-third and corn meal two-thirds, five, or 83 percent, finished.

Of six Barred Plymouth Rock hens starting on soybean meal one-third and corn meal two-thirds, five, or 83 percent, finished, thus placing both soybean and peanut meals high from a tolerance standpoint. This corresponds with our many practical feeding tests with these two feeds.

Of six Partridge Plymouth Rocks starting on twenty percent dried buttermilk and eighty percent corn meal, five, or 83 percent, finished.

Of six Buff Plymouth Rock hens starting on 20 percent dried blood and 80 percent corn, four, or 66 percent, finished.

Of six White Plymouth Rock and Buff Orpington hens starting on digester tankage 20 percent and corn meal 80 percent, two, or 33 percent, finished. This corresponds to our various series of practical feeding tests that digester tankage, at least some batches, is hard on fowls.

Of six White Plymouth Rock hens starting on beef scrap and bone meal one-third part and corn meal two-thirds part, two, or 33 percent, finished.

In a study of these tests it appears that of 105 hens that started the various tests, but 48 or 45.7 percent, finished the test. Of the 79 started on two feeds, 53, or 67.4 percent, finished the test. From a tolerance standpoint the odds are in favor of more than one feed against the single feed.

SUMMARY OF THE DIGESTIVE COEFFICIENT STUDIES

In a review of research literature we find but eighty-nine individual digestion coefficient tests with poultry. To this we have added 101, making 190 tests to date.

The digestion coefficients with poultry were studies on nineteen feeds. In the case of wheat middlings two series of tests were run with middlings alone and one in combination with unbolted corn meal. Since wheat middlings alone tends to cause looseness of the bowels, it was the desire to determine if this in any way influenced the accuracy of the work. To test this point a series of experiments were run with a combination of wheat middlings and unbolted corn meal. In the case of wheat middlings alone the average digestible organic matter is shown to be 47.72 percent and 56.19 percent with an average of 51.95 percent, while in combination with corn meal the percentage digestible organic matter was 59.78 percent.

In the experiments with corn meal one series of trials was run with bolted and another series with unbolted meal.

Not only did the birds stand up better under more than one feed but apparently one feed favored the increased digestion of the other feed. Some of the feeds, as rye and middlings, if given in their pure form cause irritation of the bowel and looseness and still others, especially those of the animal products group, as digester tankage, meat and bone meal, blood meal, dried buttermilk, and fish meal are so concentrated that it is necessary to give them with other feeds in the digestive tests. In our work we used unbolted corn meal as shown in the tables. Digestion tests had previously been run with the corn meal to determine its digestibility.

Crost in 1900 gave as his results that the crude protein of barley and rye was more digestable than that of oats. We cannot confirm this result as in two tests with barley 72.77 percent of the protein was digested, while in three tests with rye there was 71.85 percent digested and in six tests with oats there was 73.49 percent digested or slightly greater digestion of protein in oats than in either barley or rye, which is the reverse of his findings. Crost further states that the crude fiber of barley and oats was absorbed to a slight degree, but more in the case of rye. In our case 4.33 percent fiber was digested in barley and in rye 4.68 percent and in oats 11.69 per cent. In our work the fiber of oats ranked first, rye second, with barley closely following. Crost states that the availability of the fat in rye is very low as compared with the fat in oats and barley. In this we agree as the digestibility of the fat of rye was but 27.87 percent, 64.36 for barley, and 74.42 for oats.

Brown in 1904 concludes from his experiments that fowls digest crude protein and nitrogen-free-extract in much greater proportions in corn than in oats, and that wheat falls between the two. In our experiments in five tests with corn there was a total of 83.9 percent organic matter digested and with wheat in six digestion trials ranking second and oats third with 63.78 percent. As to protein, oats rank first with 73.49 percent digested, corn second with 68.71 percent, and wheat third with 61.70 per cent digested. As to nitrogen-free-extract, corn ranks first with 89.40 percent digested, oats second with 88.18 percent, and wheat third with 86.78 percent. Wheat shows a low percentage digestible fat being only 37.76 percent, and oats 74.42 percent. This latter corresponds to Brown's findings. The nutritive value of these three grains we place in the order of corn, wheat, oats. The low fat content of wheat, it being but 2.90 percent, allows of wheat being placed above oats. The order of value of all grains tested based on quantity of digestible nutrients as indicated in these tests is: corn, hulled oats, wheat, kafir corn, rye, barley, oats, buckwheat, and rice.

Our tests confirm those of Bartlett and others, that fowls digest fiber with difficulty. The percentage of fiber varies from 2.16 percent in five tests with fat extracted soybean meal to 11.69 percent in oats. It is quite evident that the fiber content of poultry feeds should be as low as possible. The rapidity of digestion in fowls may be the reason for the low digestibility of fiber. Digestion tests with livestock show that the animals with the longer intestine digest fiber more readily. The low digestibility of fat and fiber in wheat bran leads us to not favor its use as general and in such quantities as used by many poultry men.

Wheat middlings is at the foot of the list in the total digestible pounds in one hundred pounds. This is true studying the first series of nine digestion trials showing 49.99 pounds in one hundred pounds, the second series of six tests with 58.61 pounds, and the third in combination with corn meal with 61.70 pounds in each one hundred pounds. The average of these eighteen tests is 56.76 pounds.

With the exceptions of wheat middlings, meat and bone meal, digester tankage, and blood meal these tests show that fowls are more efficient in the digestion of nitrogen-free-extract than in the digestion of crude protein or fat. These include corn, wheat, oats, corn meal (bolted), buttermilk, (dried) soybean meal (fat extracted), peanut meal (fat extracted), cotton seed meal, corn meal (unbolted), rye, hulled oats, buckwheat, rice, kafir corn, and barley. They are more efficient in digesting fat over protein in corn, oats, corn meal (bolted), meat and bone meal, digester tankage, fish meal, corn meal (unbolted), hulled oats, buckwheat, rice, and kafir corn. They are more efficient in the digestion of protein over fat in wheat middlings, wheat, blood meal, buttermilk (dried), soybean meal (fat extracted), peanut meal (fat extracted), cotton seed meal, rye, and barley.

In studying the digestion results with these eighteen feeds we note that the feeds showing the highest percentage digestible matter are those low in fiber. These are among the grains corn, wheat, hulled oats, rye, and kafir corn and among the by-products corn meal and fat extracted soybean meal, and among all of the animal products. Those feeds that are relatively high in fiber, such as wheat middlings, oats, buckwheat, and rice, are relatively low in total digestible organic matter. This reflects the inability of fowls to use to advantage the coarser feed stuffs and suggests that the feed mixtures for poultry should contain relatively small proportions of such feeds as alfalfa meal, wheat bran, and wheat middlings. It is believed that rations that carry a low percentage of fiber will give the best results.

The highest percentage of digestion of protein is that of meat and bone meal with fish meal coming a close second, digester tankage ranks third, and blood meal fourth, fat extracted soybean fifth, and dried buttermilk sixth. Fowls assimilate best the protein of animal products

In our tests, numbering 13 in all, the protein of corn meal either bolted or unbolted is digested to better advantage than that of whole corn.

Our tests show 88.18 percent nitrogen-free-extract digested in oats in six tests as compared to sixty-nine by other authors with the exception of Bartlett who gives 90.10 percent. Our hulled oats experiment, five tests, shows 86.22 percent digested by fowls. The feeds high in nitrogen-free-extract can well be used to make up the starch or carbohydrate part of our poultry feeds. Such feeds are corn, wheat, oats, and kafir corn. Kafir corn makes an excellent substitute for wheat and should be used more if the price permits.

With the exception of wheat and wheat middlings the digestion of fat or ether extract is no more variable than for other feeds. Barley is only moderately low, having 64.36 percent of the fat digestible.

TABLE IV

SHOWING COMPOSITION OF POULTRY FEEDS USED IN THESE TESTS

	No. Samples	Water	Ash	Crude Protein	Carbohydrates		
					Fiber	N.F.Ext.	Fat
Wheat middlings	6	9.22	4.43	14.93	6.25	61.06	4.11
Corn	6	10.29	1.61	10.30	2.60	70.10	5.10
Wheat	6	10.10	2.0	12.50	3.10	69.40	2.90
Oats	4	11.65	2.49	11.62	12.08	59.14	3.02
Corn meal (bolted)	6	13.13	1.23	10.10	1.79	68.10	5.65
Meat and bone meal	4	4.52	30.02	38.06	2.05	11.47	13.88
Digester tankage	4	6.4	14.00	58.20	4.60	2.70	14.10
Blood meal	4	9.10	3.70	83.10	----------	3.50	0.60
Butter milk (dried)	4	10.12	6.42	34.16	----------	48.31	0.99
Soybean meal (fat extracted)	4	10.12	5.36	42.10	5.70	29.12	7.60
Peanut meal (fat not extred.)	4	6.10	4.90	21.30	16.60	15.50	35.60
Fish meal	4	9.70	31.10	49.30	----------	----------	9.90
Cottonseed meal	4	8.30	6.90	40.30	9.60	26.80	8.10
Corn meal (unbolted)	4	11.10	2.00	9.10	2.90	69.30	5.60
Wheat middlings	4	9.40	4.20	16.90	8.50	55.90	5.10
Rye	4	10.30	2.20	11.90	3.40	70.10	2.10
Hulled oats	5	8.20	3.10	13.90	1.90	64.20	8.70
Buckwheat	3	10.50	2.90	11.10	8.90	63.60	3.00
Rice	6	8.90	5.30	8.10	10.10	65.60	2.00
Kafir corn	6	11.00	2.10	10.90	3.00	69.80	3.20
Barley	4	10.10	3.20	10.90	5.00	67.90	2.90

TABLE V

SHOWING AVERAGE DIGESTIBILITY OF POULTRY FEEDS IN THESE TESTS

Feed	No. Trials	Organic Matter	Crude Protein	Fat	Carbohydrates	
					Fiber	N. F. Ext.
Wheat middlings	9	47.72	52.91	54.09	8.76	49.99
Corn	5	83.91	68.71	75.26	5.86	89.40
Wheat	6	78.50	61.70	37.76	6.40	86.78
Oats	6	63.78	73.49	74.42	11.69	88.18
Corn meal (bolted)	5	84.67	74.04	86.26	6.66	88.18
Meat and bone meal	2	86.82	92.17	93.13	3.23	76.56
Digester tankage	2	85.50	90.70	91.71	3.94	81.88
Blood meal	4	86.71	88.10	78.36	----------	71.55
Buttermilk (dried)	5	80.79	81.55	78.01	----------	81.14
Soybean meal (fat extracted)	5	77.61	83.33	81.41	2.16	83.14
Peanut meal (fat not extracted)	5	65.94	80.30	78.41	4.08	84.14
Fish meal	5	91.60	91.48	92.24	----------	----------
Cottonseed meal	4	73.32	81.94	79.05	5.46	82.92
Corn meal (unbolted)	3	83.21	73.50	84.50	6.80	87.60
Wheat middlings	6	56.19	75.43	42.64	10.20	58.61
Rye	3	77.65	71.85	27.87	4.68	83.65
Hulled oats	5	81.75	69.83	84.57	4.91	86.22
Buckwheat	6	70.39	55.93	83.73	6.33	81.25
Rice	5	69.17	73.08	80.00	5.81	78.93
Kafir corn	5	78.72	68.56	75.61	4.45	83.65
Barley	2	75.35	72.77	64.36	4.33	81.46
Wheat middlings	3	59.78	77.57	72.22	4.05	61.70

TABLE VI

SHOWING AVERAGE DIGESTIBLE NUTRIENTS IN 100 LBS. OF FEED IN THESE TESTS

Feed	Total dry Matter lbs.	Crude Protein Lbs.	Fat Lbs.	Carbohydrates		Total lbs.
				Fiber lbs.	N. F. Ext. Lbs.	
Wheat middlings	90.78	7.98	2.22	0.55	30.52	41.27
Corn	89.71	6.98	3.84	0.55	62.67	74.02
Wheat	89.90	7.71	1.09	0.19	60.22	69.21
Oats	88.35	8.53	2.24	1.41	52.14	64.32
Corn meal (bolted)	86.87	7.47	4.87	0.11	60.45	72.90
Meat and bone meal	95.48	35.07	12.92	0.07	8.78	56.84
Digester tankage	93.60	52.78	12.93	0.18	2.21	68.10
Blood meal	90.90	73.21	0.47	----------	2.50	76.18
Buttermilk (dried)	89.88	27.85	0.77	----------	39.19	67.81
Soybean meal (fat extracted)	89.88	35.08	6.18	0.12	24.21	65.59
Peanut meal (fat not extracted)	93.90	17.10	27.91	0.67	13.04	58.72
Fish meal	90.30	45.10	9.13	----------	----------	54.23
Cottonseed meal	91.70	33.02	6.40	0.52	22.22	62.16
Corn meal (unbolted)	88.90	6.67	4.73	0.20	60.71	72.31
Wheat middlings	90.60	12.75	2.17	0.87	32.76	48.55
Rye	89.70	8.55	0.59	0.16	58.64	67.94
Hulled oats	91.80	9.71	7.36	0.09	55.35	72.51
Buckwheat	89.50	6.21	2.51	0.36	51.68	60.96
Rice	91.10	5.92	1.60	0.64	51.78	59.94
Kafir corn	89.00	7.47	2.42	0.13	58.47	68.49
Barley	89.90	7.93	1.87	0.22	55.31	65.33
Wheat middlings	90.60	13.11	3.68	0.34	34.49	51.62

KEY TO TABLES

The letter of the alphabet preceding the numeral refers to the feed used, the numeral refers to the hen number used in the test and also the excrement number. Thus A-1 would mean wheat middlings, hen No. 1 and excrement No. 1.

A—Wheat middlings.
B—Corn.
C—Wheat.
D—Oats.
E—Corn meal (bolted).
F—Beef scrap 1-3, corn meal 2-3.
G—Tankage 20%, corn meal 80%.
H—Blood meal 20%, corn meal 80%.
I—Buttermilk (dried) 20%, corn meal 80%.
J—Soybean meal (fat extracted) 1-3, corn meal 2-3.
K—Peanut meal (fat extracted) 1-3, corn meal 2-3.
M—Fish meal 20%, corn meal 80%.
N—Cottonseed meal 1-3, corn meal 2-3.
O—Corn meal (unbolted).
P—Wheat middlings.
Q—Rye ¼, corn meal ¾.
R—Hulled oats.
S—Buckwheat ¼, corn meal ¾.
T—Rice ¼, corn meal ¾.
U—Kafir corn ¼, corn meal ¾.
V—Barley ¼, corn meal ¾.
W—Wheat middlings ¼, corn meal ¾.

FEEDING DATA

HEN NO. 301. BREED: WHITE P. ROCK. COOP NO. 1. KIND OF FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	10	10	20	30	------------	6.0	Laid
Second day	10	12	22	33	------------	6.0	------------
Third day	12	15	27	40.5	------------	5.9	------------
Fourth day	15	15	30	45	------------	5.9	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	12	12	24	36	18.5	5.8	------------
Second day	12	12	24	36	18.7	5.8	------------
Third day	12	12	24	36	15	5.7	------------
Fourth day	12	12	24	36	20.5	5.7	------------
Totals	48	48	96	144	72.7	------------	------------

Excrement No. A-1.

HEN NO. B36. BREED: WHITE P. ROCK. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	7	10	17	34	------------	5.2	Laid Blood-like Discharge.
Second day	10	12	22	44	------------	5.2	------------
Third day	12	14	26	52	------------	5.2	------------
Fourth day	15	15	30	60	------------	5.2	Laid.

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	12	12	24	36	18.0	5.2	------------
Second day	12	12	24	36	18.5	5.2	------------
Third day	12	12	24	36	17.5	5.2	------------
Fourth day	12	12	24	36	17.7	5.1	------------
Totals	48	48	96	144	71.7	------------	------------

Excrement No. A-2.

HEN NO. 142. COOP NO. 3. BREED: WHITE P. ROCK. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	8	12	18	36	------------	5.9	Sluggish
Second day	10	12	22	44	------------	5.9	------------
Third day	12	12	24	48	------------	5.9	------------
Fourth day	12	12	24	48	------------	5.9	Laid

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	12	10	22	33	10.7	5.9	------------
Second day	8	9	16	24	19.7	5.8	------------
Third day	12	12	24	36	19	5.8	------------
Fourth day	12	12	24	36	17.2	5.8	------------
Totals	44	44	88	129	66.6	------------	------------

Excrement No. A-3.

HEN NO. 150. BREED: WHITE PLYMOUTH ROCK. COOP NO. 1. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	10	11	21	42	------------	5.9	------------
Second day	15	15	30	60	------------	5.9	------------
Third day	20	15	35	70	------------	5.9	------------
Fourth day	12	10	22	44	------------	5.9	Laid

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	11	12	23	34.5	17	5.9	------------
Second day	15	15	30	45	18.5	5.9	------------
Third day	15	10	25	37.5	18	5.8	------------
Fourth day	15	12	27	40.5	16.5	5.8	------------
Totals	56	49	105	157.5	70.0	------------	------------

Excrement No. A-4.

HEN NO. 28. BREED: BUFF ROCK. COOP NO. 2. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	10	11	22	44	------------	5.3	------------
Second day	15	15	30	60	------------	5.3	------------
Third day	20	15	35	70	------------	5.2	------------
Fourth day	15	15	30	60	------------	5.2	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	12	12	24	36	17	5.0	------------
Second day	15	15	30	45	17.5	5.0	Laid.
Third day	15	15	30	45	15.5	4.9	------------
Fourth day	15	15	30	45	24.5	4.9	------------
Totals	57	57	114	171	74.5	------------	------------

Excrement No. A-5.

HEN NO. 473. BREED: BUFF P. ROCK. COOP NO. 3. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	10	11	22	44	------------	5.5	------------
Second day	15	15	30	60	------------	5.5	Laid.
Third day	20	15	35	70	------------	5.3	------------
Fourth day	15	15	30	60	------------	5.3	Laid.

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	12	12	24	36	20	5.2	------------
Second day	15	15	30	45	22	5.2	------------
Third day	15	15	30	45	15	5.2	------------
Fourth day	15	15	30	45	21	5.2	------------
Totals	57	57	114	171	78	------------	------------

Excrement No. A-6

HEN NO. 152. BREED: BUFF ORPINGTON. COOP NO. 1. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	90	------------	7.7	------------
Second day	20	20	40	120	------------	7.7	------------
Third day	20	20	40	120	------------	7.6	Laid.
Fourth day	25	30	55	165	------------	7.5	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	150	42.5	7.5	------------
Second day	30	30	60	150	40.0	7.5	------------
Third day	30	30	60	150	38	7.4	------------
Fourth day	30	30	60	150	30	7.4	------------
Totals	120	120	240	660	150	------------	------------

Excrement No. A-7.

HEN NO. 238. BREED: BUFF ORPINGTON. COOP NO. 2. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	90	------------	7.3	------------
Second day	20	20	40	120	------------	7.3	------------
Third day	20	20	40	120	------------	7.3	Laid.
Fourth day	25	30	55	165	------------	7.2	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	44.5	7.1	Laid.
Second day	30	30	60	150	30.5	6.9	------------
Third day	30	30	60	180	37.0	6.9	------------
Fourth day	30	30	60	180	35.0	6.8	------------
Totals	120	120	240	660	146	------------	------------

Excrement No. A-8.

HEN NO. 171. BREED: BUFF ORPINGTON. COOP NO. 3. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	90	------------	6.1	Hen in laying condition, but somewhat sluggish.
Second day	20	20	40	120	------------	6.1	
Third day	20	20	40	120	------------	6.1	
Fourth day	25	25	50	150	------------	6.0	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	25	25	50	125	35.0	6.0	------------
Second day	20	20	40	------------	34.5	5.9	------------
Third day	25	30	55	145	28.0	5.9	------------
Fourth day	30	30	60	180	35.0	5.8	------------
Total	100	105	205	575	132	------------	------------

Excrement No. A-9.

HEN NO. 10. BREED: BUFF ROCK. COOP NO. 1. FEED: CORN.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	------------	6.5	------------
Second day	20	20	40	80	------------	6.5	------------
Third day	20	20	40	80	------------	6.5	------------
Fourth day	20	20	40	80	------------	6.5	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	20	40	80	8.0	6.5	------------
Second day	20	20	40	80	7.0	6.5	------------
Third day	20	20	40	80	5.0	6.6	------------
Fourth day	20	20	40	80	9.0	6.6	------------
Totals	80	80	160	320	29	------------	------------

Excrement No. B-1.

Hen No. 44. Breed: Buff Rock. Coop No. 2. Feed: Corn.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	------------	4.6	------------
Second day	20	20	40	80	------------	4.6	Laid.
Third day	20	20	40	80	------------	4.6	------------
Fourth day	20	20	40	80	------------	4.6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	20	40	80	8	4.6	------------
Second day	20	20	40	80	7.5	4.6	------------
Third day	20	20	40	80	6	4.8	------------
Fourth day	20	20	40	80	9	4.8	------------
Totals	80	80	160	320	30.5	------------	------------

Excrement No. B-2.

Hen No. 150. Breed: Buff Plymouth Rock. Coop No. 3. Feed: Corn.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	------------	5.0	Laid.
Second day	20	20	40	80	------------	5.0	Laid.
Third day	20	20	40	80	------------	4.9	------------
Fourth day	20	20	40	80	------------	4.8	Laid.

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	20	40	80	10	4.6	------------
Second day	20	20	40	80	9	4.6	Laid.
Third day		Refused to eat.				4.5	------------
Fourth day		Refused to eat.				4.4	------------
Totals	40	40	80	160	19	------------	------------

Excrement No. B-3.

HEN NO. 179. BREED: BUFF P. ROCK. COOP NO. 4. FEED: CORN.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	------------	4.4	------------
Second day	20	20	40	80	------------	4.4	------------
Third day	20	20	40	80	------------	4.4	------------
Fourth day	20	20	40	80	------------	4.4	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	20	40	80	8	4.3	------------
Second day	20	20	40	80	8	4.3	------------
Third day	20	20	40	80	7	4.3	------------
Fourth day	20	20	40	80	6	4.3	------------
Totals	80	80	160	320	29	------------	------------

Excrement No. B-4.

HEN NO. 148. BREED: BUFF P. ROCK. COOP NO. 6. FEED: CORN.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	------------	4.6	Laid.
Second day	20	20	40	80	------------	4.6	------------
Third day	20	20	40	80	------------	4.6	------------
Fourth day	20	20	40	80	------------	4.6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	20	40	80	7	4.6	------------
Second day	20	20	40	80	8	4.6	------------
Third day	20	20	40	80	9	4.6	------------
Fourth day	20	15	35	70	6	4.5	------------
Totals	80	75	155	210	30	------------	------------

Excrement No. B-6.

Hen No. xx. Breed: White P. Rock. Coop No. 1. Feed: Wheat.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	------------	5.4	Laid.
Second day	25	20	35	70	------------	5.4	------------
Third day	20	20	40	80	------------	5.5	------------
Fourth day	20	20	40	80	------------	5.5	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	20	40	80	10	5.5	Laid.
Second day	20	20	40	80	12	5.5	------------
Third day	20	20	40	80	8	5.5	------------
Fourth day	20	20	40	80	11	5.4	------------
Totals	80	80	160	320	41	------------	------------

Excrement No. C-1.

Hen No. 00. Breed: White P. Rock. Coop No. 2. Feed: Wheat.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	------------	5.2	Laid.
Second day	15	20	35	70	------------	5.2	------------
Third day	15	5	20	40	------------	5.1	Laid.
Fourth day	10	10	20	40	------------	5.0	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	10	5.0	------------
Second day	20	20	40	80	13.5	5.0	------------
Third day	20	20	40	80	8.0	5.1	------------
Fourth day	20	20	40	80	9.0	5.1	------------
Totals	75	75	150	300	40.5	------------	------------

Excrement No. C-2.

HEN NO. 20. BREED: WHITE P. ROCK. COOP NO. 3. FEED: WHEAT.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60		6.3	Laid.
Second day	20	20	40	80		6.3	Laid.
Third day	20	20	40	80		6.2	
Fourth day	20	20	40	80		6.1	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	20	40	80	10	6.1	
Second day	20	20	40	80	15	6.2	
Third day	20	20	40	80	11.5	6.2	
Fourth day	20	20	40	80	10	6.2	
Totals	80	80	120	240	46.5		

Excrement No. C-3.

HEN NO. 60. BREED: WHITE P. ROCK. COOP NO. 4. FEED: WHEAT.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60		4.3	
Second day	25	20	45	90		4.3	
Third day		15	15	30		4.2	
Fourth day	10	10	20	40		4.1	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	10	25	50	7	4.0	
Second day	20	20	40	80	10	4.0	
Third day	20	20	40	80	15	4.0	
Fourth day	20	20	40	80	9.5	4.0	
Totals	75	70	145	290	41.5		

Excrement No. C-4.

Hen No. 71. Breed: White P. Rock. Coop No. 5. Feed: Wheat.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	------------	4.9	Laid.
Second day	20	20	40	80	------------	4.8	------------
Third day	20	20	40	80	------------	4.7	Laid.
Fourth day	20	20	40	80	100	4.6	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	20	40	80	10	4.5	------------
Second day	20	20	40	80	10	4.5	------------
Third day	20	20	40	80	10	4.6	------------
Fourth day	20	20	40	80	10	4.6	------------
Totals	80	80	160	320	40	------------	------------

Excrement No. C-5.

Hen No. 72. Breed: White P. Rock. Coop No. 6. Feed: Wheat.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	15	15	30	60	------------	6.3	Laid.
Second day	20	20	40	80	------------	6.3	------------
Third day	20	20	40	80	------------	6.2	Laid.
Fourth day	20	20	40	80	------------	6.1	Laid.

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	20	40	80	5	6.1	Laid.
Second day	20	20	40	80	17	6.2	------------
Third day	20	20	40	80	10	6.2	------------
Fourth day	20	20	40	80	12	6.1	------------
Totals	80	80	160	320	44	------------	------------

Excrement No. C-6.

HEN NO. 1. COOP NO. 1. BREED: BUFF ORPINGTON. KIND OF FEED: OATS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	35	65	------------	------------	6.3	Feed mixed with twice the amount of distilled water.
Second day	30	30	60	------------	------------	6.3	
Third day	30	30	60	------------	------------	6.2	
Fourth day	30	30	60	------------	------------	6.2	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	
First day	30	30	60	------------	18	6.1	Bronchitis The oats had to be finely ground in order to keep hens from going off feed.
Second day	30	30	60	------------	25	6.1	
Third day	30	30	60	------------	32	6.0	
Fourth day	30	30	60	------------	33	6.0	
Total	120	120	240	------------	108	------------	

Excrement No. D-7.

HEN NO. 1. BREED: BUFF ORPINGTON. COOP NO. 2. FEED: OATS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	------------	------------	6.0	------------
Second day	28	30	58	------------	------------	6.0	------------
Third day	30	30	60	------------	------------	6.0	------------
Fourth day	30	30	60	------------	------------	6.0	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	------------	20	5.9	Laid today.
Second day	30	30	60	------------	16	5.9	------------
Third day	30	30	60	------------	31	5.9	Laid today.
Fourth day	30	30	60	------------	30	5.8	------------
Total	120	120	240	------------	97	------------	------------

Excrement No. D-8.

HEN NO. 3. BREED: BUFF ORPINGTON. KIND OF FEED: OATS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	----------	----------	5.6	----------
Second day	30	30	60	----------	----------	5.6	----------
Third day	30	30	60	----------	----------	5.5	----------
Fourth day	30	30	60	----------	----------	5.5	----------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	18	----------	5.4	----------
Second day	30	30	60	15	----------	5.4	----------
Third day	30	30	60	35	----------	5.3	----------
Fourth day	30	30	60	25	----------	5.3	----------
Total	120	120	240	92	----------	5.3	----------

Excrement No. D-9.

HEN NO. 4. BREED: BUFF ORPINGTON. FEED: OATS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	----------	----------	6.0	----------
Second day	30	30	60	----------	----------	6.0	----------
Third day	30	30	60	----------	----------	6.0	----------
Fourth day	30	30	60	----------	----------	5.9	----------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	----------	22	5.8	----------
Second day	30	30	60	----------	18	5.7	----------
Third day	30	30	60	----------	23	5.7	----------
Fourth day	30	30	60	----------	29	5.7	----------
Total	120	120	240	----------	92	----------	----------

Excrement No. D-10.

HEN NO. 5. BREED: BUFF ORPINGTON. COOP NO. 5. FEED: OATS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60			5.4	
Second day	30	30	60			5.4	
Third day	30	30	60			5.3	
Fourth day	30	30	60			5.3	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60		20	5.2	
Second day	30	30	60		20	5.1	
Third day	30	30	60		19	5.0	
Fourth day	30	30	60		28	5.0	
Total	120	120	240		87		

Excrement No. D-11.

HEN NO. 6. BREED: BUFF ORPINGTON. COOP NO. 6. FEED: OATS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60			6.1	
Second day	30	30	60			6.0	
Third day	30	30	60			6.0	
Fourth day	30	30	60			6.0	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60		21	6.0	
Second day	30	30	60		22	6.0	
Third day	30	30	60		22	6.0	
Fourth day	30	30	60		23	6.0	
Total	120	120	240		89		

Excrement No. D-12.

Hen No. 388. Breed: Buff Rock. Coop No. 2. Feed: Corn Meal.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	------------	------------	5.5	Laid.
Second day	30	30	60	------------	------------	5.4	------------
Third day	30	35	65	------------	------------	5.3	Laid.
Fourth day	35	35	70	------------	------------	5.2	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	35	------------	35	70	10	5.2	Laid.
Second day	40	35	75	150	15	5.2	------------
Third day	35	35	70	140	14	5.2	------------
Fourth day	40	35	75	150	15	5.1	------------
Total	150	105	255	510	54	------------	------------

Excrement No. E-2.

Hen No. 149. Breed: Buff Rock. Coop No. 3. Feed: Corn Meal.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.9	------------
Second day	30	30	60	120	------------	4.7	------------
Third day	30	35	65	130	------------	4.6	------------
Fourth day	35	35	70	140	------------	4.6	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	35	------------	35	70	10	4.6	------------
Second day	40	35	75	150	14.5	4.6	------------
Third day	35	35	70	140	11	4.6	------------
Fourth day	40	35	75	150	15	4.6	------------
Total	150	105	255	510	50.5	------------	------------

Excrement No. E-3.

HEN NO. 203. BREED: BUFF ROCK. COOP NO. 5. FEED: CORN MEAL.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.4	Laid.
Second day	30	20	50	100	------------	6.3	------------
Third day	30	35	65	130	------------	6.3	------------
Fourth day	35	35	70	140	------------	6.3	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	35	------------	35	70	7	6.3	------------
Second day	40	35	75	150	10.5	6.3	------------
Third day	35	35	70	140	14	6.3	------------
Fourth day	40	35	75	150	10	6.2	------------
Total	150	105	255	510	41.5	------------	------------

Excrement No. E-5.

HEN NO. 120. BREED: WHITE ROCK. COOP NO. 2. FEED: CORN MEAL.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	35	65	130	------------	4.5	------------
Second day	35	35	70	140	------------	4.5	------------
Third day	35	35	70	140	------------	4.4	------------
Fourth day	35	30	65	130	------------	4.4	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	4.4	------------
Second day	30	30	60	120	10	4.5	------------
Third day	28	30	58	116	10	4.4	------------
Fourth day	28	30	58	116	11	4.4	------------
Total	116	120	236	472	45	------------	------------

Excrement No. E-8.

Hen No. 73. Breed: White Rock. Coop No. 5. Feed: Corn Meal.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	35	35	70	140	------------	4.7	------------
Second day	35	35	70	140	------------	4.7	------------
Third day	35	35	70	140	------------	4.7	------------
Fourth day	30	30	60	120	------------	4.7	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	13	4.7	------------
Second day	30	30	60	120	8	4.6	------------
Third day	30	30	60	120	10	4.5	------------
Fourth day	30	30	60	120	12	4.5	------------
Total	120	120	240	480	43	------------	------------

Excrement No. E-11.

Hen No. 374. Breed: Buff Rock. Coop No. 4. Feed: Corn Meal.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	35	35	70	140	------------	4.8	------------
Second day	35	35	70	140	------------	4.7	------------
Third day	35	30	65	130	------------	4.7	------------
Fourth day	30	30	60	120	------------	4.6	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	13	4.6	------------
Second day	10	0	10	100	10	4.6	Laid.
Third day	------------	------------	------------	200	------------	4.5	Off feed.
Fourth day	------------	------------	------------	200	------------	4.4	------------
Total	40	30	70	620	23	------------	------------

Excrement No. E-10.

HEN NO. 1. BREED: WHITE ROCK. COOP NO. 1. FEED: CORN MEAL 2-3, MEAT SCRAP AND BONE MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.4	------------
Second day	30	30	60	120	------------	6.4	------------
Third day	30	30	60	120	------------	6.4	------------
Fourth day	30	30	60	120	------------	6.5	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	17	6.5	------------
Second day	30	30	60	120	17	6.5	------------
Third day	30	30	60	120	12	6.5	Shows signs of constipation.
Fourth day	30	30	60	120	11.5	6.5	
Total	120	120	240	480	57.5	------------	----------

Excrement No. F-1.

HEN NO. 2. BREED: WHITE ROCK. COOP NO. 5. FEED: CORN MEAL 2-3, MEAT SCRAP AND BONE MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.1	------------
Second day	30	30	60	120	------------	6.1	------------
Third day	30	30	60	120	------------	6	------------
Fourth day	30	30	60	120	------------	6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	12	6	Began to get tired of feed.
Second day	30	30	60	120	17	6	
Third day	30	30	60	120	12	6	
Fourth day	30	30	60	120	13	6	------------
Total	120	120	240	480	54	------------	------------

Excrement No. F-5.

HEN NO. 230. BREED: WHITE ROCK. COOP NO. 1. FEED: CORN MEAL 80%, TANKAGE 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.7	------------
Second day	30	30	60	120	------------	4.7	------------
Third day	25	30	55	110	------------	4.6	------------
Fourth day	30	30	60	120	------------	4.6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	20	4.6	------------
Second day	30	30	60	120	18	4.6	------------
Third day	30	30	60	120	14	4.5	------------
Fourth day	30	30	60	120	19	4.4	------------
Total	120	120	240	480	71	------------	------------

Excrement No. G-1.

HEN NO. 2. BREED: WHITE ROCK. COOP NO. 2. FEED: CORN MEAL 80%, TANKAGE 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remark
First day	30	30	60	120	------------	6.4	------------
Second day	30	30	60	120	------------	6.4	------------
Third day	30	30	60	120	------------	6.3	------------
Fourth day	25	30	55	110	------------	6.3	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	19	6.2	------------
Second day	30	0	30	60	19	6.2	------------
Third day	30	30	60	120	9	6.2	------------
Fourth day	30	30	60	120	19	6.2	------------
Total	120	90	210	420	66	------------	------------

Excrement No. G-2.

HEN NO. 1. BREED: BUFF ROCK. COOP NO. 1. FEED: CORN MEAL 80%, BLOOD MEAL 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.6	------------
Second day	30	30	60	120	------------	5.5	------------
Third day	30	30	60	120	------------	5.4	------------
Fourth day	30	30	60	120	------------	5.4	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	------------	30	60	10	5.4	------------
Second day	30	30	60	120	10	5.4	------------
Third day	30	30	60	120	14	5.3	------------
Fourth day	30	30	60	120	15	5.3	------------
Total	120	90	210	420	49	------------	------------

Excrement No. H-1.

HEN NO. 2. BREED: BUFF ROCK. COOP NO. 3. FEED: CORN MEAL 80%, BLOOD MEAL 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.3	------------
Second day	30	0	30	60	------------	6.3	------------
Third day	30	30	60	120	------------	6.2	------------
Fourth day	30	30	60	120	------------	6.2	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	20	6.1	------------
Second day	30	0	30	60	11	6.1	------------
Third day	30	0	30	60	10	6.1	------------
Fourth day	0	0	------------	------------	------------	------------	------------
Total	90	30	120	240	41	------------	------------

Excrement No. H-3.

HEN NO. 3. BREED: BUFF ROCK. COOP NO. 5. FEED: CORN MEAL 80%, BLOOD MEAL 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.0	------------
Second day	30	30	60	120	------------	6.0	------------
Third day	30	------------	30	60	------------	5.9	------------
Fourth day	30	30	60	120	------------	5.9	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	0	30	60	20	5.9	------------
Second day	30	30	60	120	12	5.9	------------
Third day	30	30	60	120	18	5.8	------------
Fourth day	30	30	60	120	19	5.8	------------
Total	120	90	210	420	69	------------	------------

Excrement No. H-5.

HEN NO. 4. BREED: BUFF ROCK. COOP NO. 6. FEED: CORN MEAL 80%, BLOOD MEAL 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.4	------------
Second day	30	30	60	120	------------	6.3	------------
Third day	30	30	60	120	------------	6.3	------------
Fourth day	30	30	60	120	------------	6.3	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	0	30	60	20	6.3	------------
Second day	30	30	60	120	15	6.3	------------
Third day	30	30	60	120	15	6.3	------------
Fourth day	30	30	60	120	17	6.3	------------
Total	120	90	210	420	67	------------	------------

Excrement No. H-6.

Hen No. 1. Breed: Partridge Rock. Feed: Corn Meal 80%, Dried Buttermilk 20%.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.4	------------
Second day	30	30	60	120	------------	5.3	------------
Third day	30	30	60	120	------------	5.3	------------
Fourth day	30	30	60	120	------------	5.2	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	5.2	------------
Second day	30	30	60	120	15	5.2	------------
Third day	30	30	60	120	12	5.2	------------
Fourth day	30	30	60	120	13	5.2	------------
Total	120	120	240	480	54	------------	------------

Excrement No. I-1.

Hen No. 2. Breed: Partridge Rock. Coop No. 2. Feed: Corn Meal 80%, Dried Buttermilk, 20%.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.1	------------
Second day	30	30	60	120	------------	6.0	------------
Third day	30	30	60	120	------------	6.0	------------
Fourth day	30	30	60	120	------------	6.0	------------

Digestion Period

Day	M. Feed	N. Feed	otal	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	5.9	------------
Second day	30	30	60	120	15	5.9	------------
Third day	30	30	60	120	15	5.9	------------
Fourth day	30	30	60	120	15	6	------------
Total	120	120	240	480	59	------------	------------

Excrement No. I-2.

HEN NO. 3. BREED: PARTRIDGE ROCK. COOP NO. 3. FEED: CORN MEAL 80%, BUTTERMILK 20 %

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.8	------------
Second day	30	30	60	120	------------	5.7	------------
Third day	30	30	60	120	------------	5.7	------------
Fourth day	0	0	------------	120	------------	5.6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	10	5.6	------------
Second day	30	30	60	120	10	5.6	------------
Third day	30	30	60	120	12	5.6	------------
Fourth day		Off Feed		120	------------	5.5	------------
Total	90	90	180	480	32	------------	------------

Excrement No. I-3.

HEN NO. 4. BREED: PARTRIDGE ROCK. COOP NO. 4. FEED: CORN MEAL 80%, DRIED BUTTERMILK, 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.4	------------
Second day	30	30	60	120	------------	6.3	------------
Third day	30	30	60	120	------------	6.2	------------
Fourth day	30	30	60	120	------------	6.2	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	12	6.2	------------
Second day	30	30	60	120	10	6.2	------------
Third day	30	30	60	120	14	6.2	------------
Fourth day	30	30	60	120	13	6.2—	------------
Total	120	120	240	480	49	------------	------------

Excrement No. I-4.

HEN NO. 5. BREED: PARTRIDGE ROCK. COOP NO. 5. FEED: CORN MEAL 80%, DRIED BUTTERMILK, 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.7	------------
Second day	30	30	60	120	------------	5.6	------------
Third day	30	30	60	120	------------	5.5	------------
Fourth day	30	30	60	120	------------	5.5	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	5.5	------------
Second day	30	30	60	120	13	5.4	------------
Third day	30	30	60	120	11	5.4	------------
Fourth day	30	30	60	120	10	5.5	------------
Total	120	120	240	480	49	------------	------------

Excrement No. I-5.

HEN NO. 1. BREED: BARRED P. ROCK. COOP NO. 1. FEED: CORN MEAL 2-3, SOYBEAN MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.1	------------
Second day	30	30	60	120	------------	5.1	Laid.
Third day	30	30	60	120	------------	5.1	------------
Fourth day	30	30	60	120	------------	5.1	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	20	5.1	------------
Second day	30	30	60	120	22	5	------------
Third day	30	30	60	120	23	5	------------
Fourth day	30	30	60	120	19	5	------------
Total	120	120	240	480	84	------------	------------

Excrement No. J-1.

HEN NO. 2. BREED: BARRED P. ROCK. COOP NO. 3. FEED: CORN MEAL 2-3, SOYBEAN MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.4	------------
Second day	30	30	60	120	------------	5.4	Laid.
Third day	30	30	60	120	------------	5.4	------------
Fourth day	30	30	60	120	------------	5.4	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	20	5.4	Laid.
Second day	30	30	60	120	23	5.4	------------
Third day	30	30	60	120	22	5.3	------------
Fourth day	30	30	60	120	19	5.3	------------
Total	120	120	240	480	84	------------	------------

Excrement No. J-3.

HEN NO. 3. BREED: BARRED P. ROCK. COOP NO. 4. FEED: CORN MEAL 2-3, SOYBEAN MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.0	------------
Second day	30	30	60	120	------------	5.0	------------
Third day	30	30	60	120	------------	5.0	------------
Fourth day	30	30	60	120	------------	4.9	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	22	4.9	------------
Second day	30	30	60	120	25	4.9	------------
Third day	30	20	50	110	26	4.8	------------
Fourth day		Off feed		120	------------	4.8	------------
Total	90	80	170	470	73	------------	------------

Excrement No. J-4.

HEN NO. 4. BREED: BARRED P. ROCK. COOP NO. 5. FEED: CORN MEAL 2-3, SOYBEAN MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.0	------------
Second day	30	30	60	120	------------	6	------------
Third day	30	30	60	120	------------	6.0	------------
Fourth day	30	30	60	120	------------	6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	25	6	------------
Second day	30	30	60	120	18	6	------------
Third day	30	30	60	120	22	6 +	------------
Fourth day	30	30	60	120	20	6.1	------------
Total	120	120	240	480	85	------------	------------

Excrement No. J-5.

HEN NO. 5. BREED: BARRED P. ROCK. COOP NO. 6. FEED: CORN MEAL 2-3, SOYBEAN MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.7	------------
Seocnd day	30	30	60	120	------------	5.7	------------
Third day	30	30	60	120	------------	5.7	------------
Fourth day	30	30	60	120	------------	5.7	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	22	5.7	------------
Second day	30	30	60	120	23	5.6	------------
Third day	30	30	60	120	22	5.6	------------
Fourth day	30	30	60	120	20	5.5	------------
Total	120	120	240	480	87	------------	------------

Excrement No. J-6.

HEN NO. 1. BREED: BARRED PLYMOUTH ROCK. COOP NO. 1. FEED: CORN MEAL 2-3, PEANUT MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Exerement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.7	------------
Second day	30	30	60	120	------------	5.7	------------
Third day	30	30	60	120	------------	5.6	------------
Fourth day	30	30	60	120	------------	5.6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	17	5.5	------------
Second day	30	30	60	120	18	5.5	------------
Third day	30	30	60	120	16	5.5	------------
Fourth day	30	30	60	120	19	5.5	------------
Total	120	120	240	480	70	------------	------------

Exerement No. K-1.

HEN NO. 2. BREED: BARRED PLYMOUTH ROCK. COOP NO. 2. FEED: CORN MEAL 2-3, PEANUT MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Exerement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.9	------------
Second day	30	30	60	120	------------	4.9	------------
Third day	30	30	60	120	------------	4.9	------------
Fourth day	30	30	60	120	------------	4.8	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	16	4.8	------------
Second day	30	30	60	120	19	4.8	------------
Third day	30	30	60	120	20	4.8	------------
Fourth day	30	30	60	120	15	4.7	------------
Total	120	120	240	480	70	------------	------------

Exerement No. K-2.

HEN NO. 3. BREED: BARRED PLYMOUTH ROCK. COOP NO. 3. FEED: CORN MEAL 2-3, PEANUT MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day ------	30	30	60	120	------------	5.1	------------
Second day ----	30	30	60	120	------------	5.0	------------
Third day -----	30	30	60	120	------------	5.0	------------
Fourth day ----	30	30	60	120	------------	5.0	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day ------	30	30	60	120	20	5.0	------------
Second day ----	30	30	60	120	15	5.0	------------
Third day -----	30	30	60	120	18	5.0	------------
Fourth day ----	30	30	60	120	16	4.9	------------
Total ----------	120	120	240	480	69	------------	------------

Excrement No. K-3.

HEN NO. 4. BREED: BARRED PLYMOUTH ROCK. COOP NO. 5. FEED: CORN MEAL 2-3, PEANUT MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day ------	30	30	60	120	------------	5.3	------------
Second day ----	30	30	60	120	------------	5.3	------------
Third day -----	30	30	60	120	------------	5.3	------------
Fourth day ----	30	30	60	120	------------	5.3	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day ------	30	30	60	120	23	5.2	------------
Second day ----	30	30	60	120	16	5.1	------------
Third day -----	30	30	60	120	20	5.1	------------
Fourth day ----	30	30	60	120	25	5.2	------------
Total ----------	120	120	240	480	84	------------	------------

Excrement No. K-5.

HEN NO. 5. BREED: BARRED PLYMOUTH ROCK. COOP NO. 6. FEED: CORN MEAL 2-3, PEANUT MEAL 1-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120		4.6	
Second day	30	30	60	120		4.5	
Third day	30	30	60	120		4.5	
Fourth day	30	30	60	120		4.4	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	19	4.4	
Second day	30	30	60	120	22	4.4	
Third day	30	30	60	120	20	4.4	
Fourth day	30	30	60	120	25	4.4	
Total	120	120	240	480	86		

Excrement No. K-6.

HEN NO. 1. BREED: RHODE ISLAND RED. COOP NO. 1. FEED: CORN MEAL 80%, FISH MEAL 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120		4.6	
Second day	30	30	60	120		4.6	
Third day	30	30	60	120		4.6	
Fourth day	30	30	60	120		4.6	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	4.6	
Second day	30	30	60	120	15	4.6	
Third day	30	28	58	116	15.5	4.6	
Fourth day	30	30	60	120	14	4.6	
Total	120	118	238	476	58.5		

Excrement No. M-1.

HEN NO. 2. BREED: RHODE ISLAND RED. COOP NO. 2. FEED: CORN MEAL 80%, FISH MEAL 20%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.7	------------
Second day	30	30	60	120	------------	4.7	------------
Third day	30	30	60	120	------------	4.7	------------
Fourth day	30	30	60	120	------------	4.7	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	25	55	110	13	4.8	------------
Second day	30	30	60	120	12.5	4.8	------------
Third day	30	30	60	120	16	4.8	------------
Fourth day	30	30	60	120	14	4.8	------------
Total	120	115	235	470	55	------------	------------

Excrement No. M-2.

HEN NO. 3. BREED: RHODE ISLAND RED. COOP NO. 3. FEED: FISH MEAL 20%, CORN MEAL 80%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.2	------------
Second day	30	30	60	120	------------	4.2	------------
Third day	30	30	60	120	------------	4.2	------------
Fourth day	30	30	60	120	------------	4.2—	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	4.2-	------------
Second day	30	30	60	120	16	4.2-	------------
Third day	30	30	60	120	14	4.2-	------------
Fourth day	30	30	60	120	15	4.1	------------
Total	120	120	240	480	60	------------	------------

Excrement No. M-3.

HEN NO. 4. BREED: RHODE ISLAND RED. COOP NO. 5. FEED: FISH MEAL 20%, CORN MEAL 80%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.3	------------
Second day	30	30	60	120	------------	4.3	------------
Third day	30	30	60	120	------------	4.3	------------
Fourth day	30	30	60	120	------------	4.3	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	4.3	------------
Second day	30	30	60	120	14	4.3	------------
Third day	30	30	60	120	16	4.3	------------
Fourth day	30	30	60	120	14	4.3	------------
Totals	120	120	240	480	59	------------	------------

Excrement No. M-5.

HEN NO. 5. BREED: RHODE ISLAND RED. COOP NO. 6. FEED: FISH MEAL 20%, CORN MEAL 80%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.5	------------
Second day	30	30	60	120	------------	4.5	------------
Third day	30	30	60	120	------------	4.5	------------
Fourth day	30	30	60	120	------------	4.5	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	4.5	------------
Second day	30	30	60	120	12	4.4	------------
Third day	30	30	60	120	14	4.4	------------
Fourth day	30	30	60	120	13	4.4	------------
Totals	120	120	240	480	54	------------	------------

Excrement No. M-6.

Hen No. 1. Breed: Golden Wyandotte. Coop No. 1. Feed: Cottonseed Meal 1-3, Corn Meal 2-3

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.3	------------
Second day	30	30	60	120	------------	5.3	------------
Third day	30	30	60	120	------------	5.3	------------
Fourth day	30	30	60	120	------------	5.3	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	20	5.3	------------
Second day	30	30	60	120	25	5.3	------------
Third day	30	30	60	120	18	5.3	------------
Fourth day	30	30	60	120	10	5.3	------------
Totals	120	120	240	480	73	------------	------------

Excrement No. N-1.

Hen No. 2. Breed: Golden Wyandotte. Coop No. 3. Feed: Cottonseed Meal 1-3, Corn Meal 2-3

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	25	55	110	------------	6.0	------------
Second day	30	30	60	120	------------	6.0	------------
Third day	30	20	50	100	------------	6.1	------------
Fourth day	30	30	60	120	------------	6.1	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	23	6.1	------------
Second day	30	30	60	120	25	6.1	------------
Third day	30	30	60	120	14	6.1	------------
Fourth day	30	30	60	120	16	6.0	------------
Totals	120	120	240	480	78	------------	------------

Excrement No. N-3.

HEN NO. 3. BREED: GOLDEN WYANDOTTE. COOP NO. 4. FEED: COTTONSEED MEAL 1-3, CORN MEAL 2-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	25	30	55	110	------------	4.9	------------
Second day	30	30	60	120	------------	4.9	------------
Third day	30	30	60	120	------------	4.9	------------
Fourth day	20	30	50	100	------------	4.9	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	22	4.9	------------
Second day	30	30	60	120	27	5.0	------------
Third day	30	30	60	120	15	5.0	------------
Fourth day	30	30	60	120	12	5.0	------------
Totals	120	120	240	480	76	------------	------------

Excrement No. N-4.

HEN NO. 4. BREED: GOLDEN WYANDOTTE. COOP NO. 6. FEED: COTTONSEED MEAL 1-3, CORN MEAL 2-3.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.7	------------
Second day	30	30	60	120	------------	5.7	------------
Third day	30	30	60	120	------------	5.7	------------
Fourth day	30	30	60	120	------------	5.7	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	25	5.7	------------
Second day	30	30	60	120	25	5.7	------------
Third day	30	30	60	120	18	5.7	------------
Fourth day	30	30	60	120	10	5.8	------------
Totals	120	120	240	480	78	------------	------------

Excrement No. N-6.

HEN NO. 1. BREED: BARRED PLYMOUTH ROCK. COOP NO. 2. FEED: CORN MEAL (UNBOLTED)

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Exerement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.7	------------
Second day	30	30	60	120	------------	------------	------------
Third day	30	30	60	120	------------	------------	------------
Fourth day	30	30	60	120	------------	------------	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Exerement	Wt. Hen	Remarks
First day	30	30	60	120	11	5.6	------------
Second day	30	30	60	120	12	5.6	------------
Third day	30	30	60	120	18	5.6	------------
Fourth day	30	30	60	120	12	5.6	------------
Totals	120	120	240	480	53	------------	------------

Excrement No. O-2.

HEN NO. 2. BREED: BARRED PLYMOUTH ROCK. COOP NO. 3. FEED: CORN MEAL (UNBOLTED).

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Exerement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.0	------------
Second day	30	30	60	120	------------	------------	------------
Third day	30	30	60	120	------------	------------	------------
Fourth day	30	30	60	120	------------	------------	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Exerement	Wt. Hen	Remarks
First day	30	30	60	120	12	5.1	------------
Second day	30	30	60	120	10	5.1	------------
Third day	30	30	60	120	12	5.1	------------
Fourth day	30	30	60	120	19	5.1	------------
Totals	120	120	240	480	53	------------	------------

Excrement No. O-3.

HEN NO. 3. BREED: BARRED PLYMOUTH ROCK. COOP NO. 5. FEED: CORN MEAL (UNBOLTED).

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.3	------------
Second day	30	30	60	120	------------	------------	------------
Third day	30	30	60	120	------------	------------	------------
Fourth day	30	30	60	120	------------	------------	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	5.2	------------
Second day	30	30	60	120	10	5.2	------------
Third day	30	30	60	120	16	5.2	------------
Fourth day	30	30	60	120	14	5.2	------------
Totals	120	120	240	480	55	------------	------------

Excrement No. O-5.

HEN NO. 1. BREED: BUFF ORPINGTON. COOP NO. 1. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.6	------------
Second day	30	30	60	120	------------	4.6	------------
Third day	30	30	60	120	------------	4.6	------------
Fourth day	30	30	60	120	------------	4.6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	25	4.6	------------
Second day	30	30	60	120	26	4.6	------------
Third day	30	30	60	120	24	4.6	------------
Fourth day	30	30	60	120	27	4.6	------------
Totals	120	120	240	480	102	------------	------------

Excrement No. P-1.

HEN NO. 0. BREED: BARRED PLYMOUTH ROCK. COOP NO. 2. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.3	------------
Second day	30	30	60	120	------------	5.3	------------
Third day	30	30	60	120	------------	5.2	------------
Fourth day	30	30	60	120	------------	5.2	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	30	5.2	------------
Second day	30	30	60	120	30	5.2	------------
Third day	30	30	60	120	24	5.2	------------
Fourth day	30	30	60	120	25	5.2	------------
Totals	120	120	240	480	109	------------	------------

Excrement No. P-2.

HEN NO. 2. BREED: BARRED PLYMOUTH ROCK. COOP NO. 3. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.9	------------
Second day	30	30	60	120	------------	4.9	------------
Third day	30	30	60	120	------------	4.9	------------
Fourth day	30	30	60	120	------------	5.0	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	27	5.0	------------
Second day	30	30	60	120	29	5.0	------------
Third day	30	30	60	120	23	5.0	------------
Fourth day	30	30	60	120	22	5.0	------------
Totals	120	120	240	480	101	------------	------------

Excrement No. P-3.

HEN NO. 3. BREED: BARRED PLYMOUTH ROCK. COOP NO. 4. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.7	------------
Second day	30	30	60	120	------------	5.7	------------
Third day	30	30	60	120	------------	5.7	------------
Fourth day	30	30	60	120	------------	5.7	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	28	5.7	------------
Second day	30	30	60	120	26	5.7	------------
Third day	30	30	60	120	18	5.7	------------
Fourth day	30	30	60	120	21	5.7	------------
Totals	120	120	240	480	110	------------	------------

Excrement No. P-4.

HEN NO. 4. BREED: BARRED PLYMOUTH ROCK. COOP NO. 5. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.0	------------
Second day	30	30	60	120	------------	6.0	------------
Third day	30	30	60	120	------------	6.0	------------
Fourth day	30	30	60	120	------------	5.9	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	30	5.9	------------
Second day	30	30	60	120	28	5.9	------------
Third day	30	30	60	120	26	5.9	------------
Fourth day	30	30	60	120	25	5.9	------------
Totals	120	120	240	480	109	------------	------------

Excrement No. P-5.

HEN No. 5. BREED: BARRED PLYMOUTH ROCK. COOP No. 6. FEED: WHEAT MIDDLINGS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.3	------------
Second day	30	30	60	120	------------	4.3	------------
Third day	30	30	60	120	------------	4.3	------------
Fourth day	30	30	60	120	------------	4.3	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	27	4.3	------------
Second day	30	30	60	120	29	4.4	------------
Third day	30	30	60	120	28	4.4	------------
Fourth day	30	30	60	120	26	4.4	------------
Totals	120	120	240	480	110	------------	------------

Excrement No. P-6.

HEN No. 120. BREED: PARTRIDGE ROCK. COOP No. 1. FEED: RYE 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.6	------------
Second day	30	30	60	120	------------	5.6	------------
Third day	30	30	60	120	------------	5.6	------------
Fourth day	30	30	60	120	------------	5.6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	5.6	------------
Second day	30	------------	30	60	18	5.6	------------
Third day	30	30	60	120	13	5.6	------------
Fourth day	30	30	60	120	16	5.6	------------
Totals	120	90	210	420	62	------------	------------

Excrement No. Q-1.

HEN NO. 36. BREED: PARTRIDGE ROCK. COOP NO. 2. FEED: RYE 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.0	------------
Second day	30	30	60	120	------------	6.0	------------
Third day	30	30	60	120	------------	6.1	------------
Fourth day	30	30	60	120	------------	6.1	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	16	6.0	------------
Second day	30	------------	30	60	14	6.0	------------
Third day	30	30	60	120	12	6.0	------------
Fourth day	30	30	60	120	19	6.0	------------
Totals	120	90	210	420	61	------------	------------

Excrement No. Q-2.

HEN NO. 41. BREED: PARTRIDGE ROCK. COOP NO. 3. FEED: RYE 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.8	------------
Second day	30	30	60	120	------------	5.8	------------
Third day	30	30	60	120	------------	5.8	------------
Fourth day	30	30	60	120	------------	5.8	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	19	5.8	------------
Second day	30	------------	30	60	13	5.8	------------
Third day	30	30	60	120	11	5.8	------------
Fourth day	30	30	60	120	18	5.8	------------
Totals	120	90	210	420	61	------------	------------

Excrement No. Q-3.

Hen No. 81. Breed: Rhode Island Red. Coop No. 1. Feed: Hulled Oats.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120		5.2	
Second day	30	30	60	120		5.2	
Third day	30	30	60	120		5.2	
Fourth day	30	30	60	120		5.2	

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	13	5.2	
Second day	30	30	60	120	15	5.2	
Third day	30	30	60	120	17	5.2	
Fourth day	30	30	60	120	15	5.2	
Totals	120	120	240	480	60		

Excrement No. R-1.

Hen No. 13. Breed: Rhode Island Red. Coop No. 2. Feed: Hulled Oats.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120		4.6	
Second day	30	30	60	120		4.6	
Third day	30	30	60	120		4.6	
Fourth day	30	30	60	120		4.5	

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	4.5	
Second day	30	30	60	120	13	4.6	
Third day	30	30	60	120	14	4.6	
Fourth day	30	30	60	120	16	4.6	
Totals	120	120	240	480	57		

Excrement No. R-2.

HEN NO. 32. BREED: RHODE ISLAND RED. COOP NO. 3. FEED: HULLED OATS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120		5.0	
Second day	30	30	60	120		5.0	
Third day	30	30	60	120		5.0	
Fourth day	30	30	60	120		5.0	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	11	4.9	
Second day	30	30	60	120	13	4.9	
Third day	30	30	60	120	16	4.9	
Fourth day	30	30	60	120	14	4.9	
Totals	120	120	240	480	54		

Excrement No. R-3.

HEN NO. 37. BREED: RHODE ISLAND RED. COOP NO. 4. FEED: HULLED OATS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120		4.8	
Second day	30	30	60	120		4.8	
Third day	30	30	60	120		4.8	
Fourth day	30	30	60	120		4.8	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	4.9	
Second day	30	30	60	120	17	4.9	
Third day	30	30	60	120	16	4.9	
Fourth day	30	30	60	120	18	4.9	
Totals	120	120	240	480	66		

Excrement No. R-4.

HEN NO. 53. BREED: RHODE ISLAND RED. COOP NO. 5. FEED: HULLED OATS.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.3	------------
Second day	30	30	60	120	------------	4.3	------------
Third day	30	30	60	120	------------	4.3	------------
Fourth day	30	30	60	120	------------	4.3	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	17	4.3	------------
Second day	30	30	60	120	20	4.3	------------
Third day	30	30	60	120	17	4.3	------------
Fourth day	30	30	60	120	19	4.3	------------
Totals	120	120	240	480	73	------------	------------

Excrement No. R-5.

HEN NO. 11. BREED: BARRED PLYMOUTH ROCK. COOP NO. 1. FEED: BUCKWHEAT 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.2	------------
Second day	30	30	60	120	------------	5.2	------------
Third day	30	30	60	120	------------	5.2	------------
Fourth day	30	30	60	120	------------	5.2	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	12	5.1	------------
Second day	30	30	60	120	13	5.1	------------
Third day	30	30	60	120	12	5.1	------------
Fourth day	30	30	60	120	14	5.1	------------
Totals	120	120	240	480	51	------------	------------

Excrement No. S-1.

Hen No. 32. Breed: Barred Plymouth Rock. Coop No. 2. Feed: Buckwheat 25%, Corn Meal 75%

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.2	------------
Second day	30	30	60	120	------------	4.2	------------
Third day	30	30	60	120	------------	4.2	------------
Fourth day	30	30	60	120	------------	4.2	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	13	4.2	------------
Second day	30	30	60	120	13	4.2	------------
Third day	30	30	60	120	15	4.2	------------
Fourth day	30	30	60	120	15	4.2	------------
Totals	120	120	240	480	57	------------	------------

Excrement No. S-2.

Hen No. 43. Breed: Barred Plymouth Rock. Coop No. 3. Feed: Buckwheat 25%, Corn Meal 75%.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.7	------------
Second day	30	30	60	120	------------	4.7	------------
Third day	30	30	60	120	------------	4.7	------------
Fourth day	30	30	60	120	------------	4.7	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	13	4.7—	------------
Second day	30	30	60	120	12	4.6	------------
Third day	30	30	60	120	13	4.6	------------
Fourth day	30	30	60	120	14	4.6	------------
Totals	120	120	240	480	52	------------	------------

Excrement No. S-3.

HEN NO. 54. BREED: BARRED PLYMOUTH ROCK. COOP NO. 4. FEED: BUCKWHEAT 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.2	------------
Second day	30	30	60	120	------------	5.2	------------
Third day	30	30	60	120	------------	5.2	------------
Fourth day	30	30	60	120	------------	5.2	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	5.3	------------
Second day	30	30	60	120	13	5.3	------------
Third day	30	30	60	120	12	5.3	------------
Fourth day	30	30	60	120	14	5.3	------------
Totals	120	120	240	480	53	------------	------------

Excrement No. S-4.

HEN NO. 65. BREED: BARRED PLYMOUTH ROCK. COOP NO. 5. FEED: BUCKWHEAT 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	25	45	90	------------	6.0	------------
Second day	30	30	60	120	------------	6.0	------------
Third day	30	30	60	120	------------	6.0	------------
Fourth day	30	30	60	120	------------	6.0	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	6.0	------------
Second day	30	30	60	120	14	6.0	------------
Third day	30	30	60	120	12	6.0	------------
Fourth day	30	30	60	120	13	6.0	------------
Totals	120	120	240	480	53	------------	------------

Excrement No. S-5.

HEN NO. 76. BREED: BARRED PLYMOUTH ROCK. COOP NO. 6. FEED: BUCKWHEAT 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.4	------------
Second day	30	30	60	120	------------	5.4	------------
Third day	30	30	60	120	------------	5.4	------------
Fourth day	30	30	60	120	------------	5.4—	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	11	5.3	------------
Second day	30	30	60	120	10	5.3	------------
Third day	30	30	60	120	20	5.3	------------
Fourth day	30	30	60	120	14	5.3—	------------
Totals	120	120	240	480	55	------------	------------

Excrement No. S-6.

HEN NO. 87. RHODE ISLAND RED. COOP NO. 1. FEED: RICE 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.1	------------
Second day	30	30	60	120	------------	5.1	------------
Third day	30	30	60	120	------------	5.1	------------
Fourth day	30	30	60	120	------------	5.1	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	5.1	------------
Second day	30	------------	30	60	16	5.1	------------
Third day	30	30	60	120	13	5.1	------------
Fourth day	30	30	60	120	19	5.1	------------
Totals	120	90	210	420	62	------------	------------

Excrement No. T-1.

HEN NO. 98. BREED: RHODE ISLAND RED. COOP NO. 2. FEED: RICE 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.0	------------
Second day	30	30	60	120	------------	6.0–	------------
Third day	30	30	60	120	------------	5.9	------------
Fourth day	30	30	60	120	------------	5.9	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	5.9	------------
Second day	30	------------	30	60	13	5.9	------------
Third day	30	30	60	120	20	5.9	------------
Fourth day	30	30	60	120	18	5.9	------------
Totals	120	90	210	420	65	------------	------------

Excrement No. T-2.

HEN NO. 23. BREED: RHODE ISLAND RED. COOP NO. 3. FEED: RICE 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.3	------------
Second day	30	30	60	120	------------	4.3	------------
Third day	30	30	60	120	------------	4.3	------------
Fourth day	30	30	60	120	------------	4.3	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	18	4.3	------------
Second day	30	------------	30	60	16	4.4	------------
Third day	30	30	60	120	14	4.4	------------
Fourth day	30	30	60	120	13	4.4	------------
Totals	120	90	210	420	61	------------	------------

Excrement No. T-3.

HEN No. 34. BREED: RHODE ISLAND RED. COOP No. 4. FEED: RICE 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.6	------------
Second day	30	30	60	120	------------	5.6	------------
Third day	30	30	60	120	------------	5.6	------------
Fourth day	30	30	60	120	------------	5.6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	17	5.6	------------
Second day	30	------------	30	60	12	5.6	------------
Third day	30	30	60	120	16	5.6	------------
Fourth day	30	30	60	120	18	5.6	------------
Totals	120	90	210	420	63	------------	------------

Excrement No. T-4.

HEN No. 45. BREED: RHODE ISLAND RED. COOP No. 5. FEED: RICE 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.4	------------
Second day	30	30	60	120	------------	5.4	------------
Third day	30	30	60	120	------------	5.4	------------
Fourth day	30	30	60	120	------------	5.4	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	5.5	------------
Second day	30	------------	30	60	17	5.5	------------
Third day	30	30	60	120	14	5.5	------------
Fourth day	30	30	60	120	16	5.5	------------
Totals	120	90	210	420	62	------------	------------

Excrement No. T-5.

Hen No. 56. Silver Pencilled Wyandotte. Coop No. 1. Feed: Kafir Corn 25%, Corn Meal 75%.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.6	------------
Second day	30	30	60	120	------------	5.6	------------
Third day	30	------------	30	60	------------	5.6	------------
Fourth day	30	30	60	120	------------	5.6	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	5.6	------------
Second day	30	30	60	120	17	5.6	------------
Third day	30	30	60	120	13	5.6	------------
Fourth day	30	30	60	120	11	5.7	------------
Totals	120	120	240	480	55	------------	------------

Excrement No. U-1.

Hen No. 67. Breed: Silver Pencilled Wyandotte. Coop No. 2. Feed: Kafir Corn 25%, Corn Meal 75%.

Preliminary Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.6	------------
Second day	30	30	60	120	------------	4.6	------------
Third day	30	30	60	120	------------	4.6	------------
Fourth day	30	------------	30	60	------------	4.6–	------------

Digestion Period

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	17	4.5	Slightly constipated
Second day	30	30	60	120	16	4.5	
Third day	30	30	60	120	11	4.5	------------
Fourth day	30	30	60	120	12	4.5	------------
Totals	120	120	240	480	56	------------	------------

Excrement No. U-2.

HEN No. 78. BREED: SILVER PENCILLED WYANDOTTE. COOP No. 3. FEED: KAFIR CORN 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.1	------------
Second day	30	30	60	120	------------	5.1	------------
Third day	30	30	60	120	------------	5.1	Slightly constipated
Fourth day	30	------------	30	60	------------	5.1	

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	19	5.2	------------
Second day	30	30	60	120	13	5.2	------------
Third day	30	30	60	120	14	5.2	------------
Fourth day	30	30	60	120	12	5.2	------------
Totals	120	120	240	480	58	------------	------------

Excrement No. U-3.

HEN No. 89. BREED: SILVER PENCILLED WYANDOTTE. COOP No. 4. FEED: KAFIR CORN 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.9	------------
Second day	30	------------	30	60	------------	4.9	------------
Third day	30	30	60	120	------------	4.9	------------
Fourth day	30	30	60	120	------------	4.9	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	11	4.9	Constipated
Second day	30	30	60	120	19	4.9	------------
Third day	30	30	60	120	16	4.9	------------
Fourth day	30	30	60	120	13	4.9	------------
Totals	120	120	240	480	59	------------	------------

Excrement No. U-4.

HEN NO. 35. BREED: SILVER PENCILLED WYANDOTTE. COOP NO. 5. FEED: KAFIR CORN 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.0	------------
Second day	30	------------	30	60	------------	4.0	------------
Third day	30	30	60	120	------------	4.0	------------
Fourth day	30	30	60	120	------------	4.0	Slightly constipated

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	14	4.0	------------
Second day	30	30	60	120	18	4.1	------------
Third day	30	30	60	120	12	4.1	------------
Fourth day	30	30	60	120	10	4.1	------------
Totals	120	120	240	480	54	------------	------------

Excrement No. U-5.

HEN NO. 46. BREED: BUFF ORPINGTON. COOP NO. 1. FEED: BARLEY 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	5.9	------------
Second day	30	30	60	120	------------	5.9	------------
Third day	30	30	60	120	------------	5.9–	------------
Fourth day	30	30	60	120	------------	5.9–	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	5.8	------------
Second day	30	30	60	120	13	5.8	------------
Third day	30	30	60	120	16	5.8	------------
Fourth day	30	30	60	120	14	5.8	------------
Totals	120	120	240	480	58	------------	------------

Excrement No. V-1.

HEN NO. 57. BREED: BUFF ORPINGTON. COOP NO. 2. FEED: BARLEY 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	6.3	------------
Second day	30	30	60	120	------------	6.3	------------
Third day	30	30	60	120	------------	6.3	------------
Fourth day	30	30	60	120	------------	6.3	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	16	6.3	------------
Second day	30	30	60	120	14	6.3	------------
Third day	30	30	60	120	15	6.3	------------
Fourth day	30	30	60	120	17	6.3	------------
Totals	120	120	240	480	62	------------	------------

Excrement No. V-2.

HEN NO. 68. BREED: BARRED PLYMOUTH ROCK. COOP NO. 1. FEED: WHEAT MIDDLINGS 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	------------	4.7	------------
Second day	30	30	60	120	------------	4.7	------------
Third day	30	30	60	120	------------	4.7	------------
Fourth day	30	30	60	120	------------	4.7	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	15	4.7	------------
Second day	30	30	60	120	19	4.7	------------
Third day	30	30	60	120	18	4.7	------------
Fourth day	30	30	60	120	17	4.7	------------
Totals	120	120	240	480	69	------------	------------

Excrement No. W-1.

HEN NO. 79. BREED: BARRED PLYMOUTH ROCK. COOP NO. 2. FEED: WHEAT MIDDLINGS 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	20	50	100	------------	5.6	------------
Second day	15	30	45	95	------------	5.6	------------
Third day	30	30	30	120	------------	5.6	------------
Fourth day	25	30	55	110	------------	5.6	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	16	5.7	------------
Second day	30	30	60	120	18	5.7	------------
Third day	30	30	60	120	14	5.7	------------
Fourth day	30	30	60	120	20	5.7	------------
Totals	120	120	240	480	68	------------	------------

Excrement No. W-2.

HEN NO. 28. BREED: BARRED PLYMOUTH ROCK. COOP NO. 3. FEED: WHEAT MIDDLINGS 25%, CORN MEAL 75%.

PRELIMINARY PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	20	15	35	70	------------	5.9	------------
Second day	25	25	50	100	------------	5.9	------------
Third day	30	25	55	110	------------	5.9	------------
Fourth day	30	30	60	120	------------	5.9	------------

DIGESTION PERIOD

Day	M. Feed	N. Feed	Total	Water	Excrement	Wt. Hen	Remarks
First day	30	30	60	120	20	5.9	------------
Second day	30	30	60	120	14	6.0	------------
Third day	30	30	60	120	16	6.0	------------
Fourth day	30	30	60	120	17	6.0	------------
Totals	120	120	240	480	67	------------	------------

Excrement No. W-3.

EXCREMENT ANALYSIS

	Excrement A-1	Excrement A-2	Excrement A-3
Moisture	10.91	10.37	10.55
Total nitrogen	6.01	6.03	6.01
Uric acid nitrogen	3.78	3.72	3.69
Ammonia nitrogen	.64	.57	.60
Ether extract	2.75	2.64	2.56
Crude fiber	7.49	8.44	7.10
Nitrogen free extract	45.21	45.67	44.18
Uric acid	11.46	11.29	11.08
Ammonia	.80	.70	.73
Ash	11.44	10.05	13.05
Crude protein	9.94	10.84	10.74

	Excrement A-4	Excrement A-5	Excrement A-6
Moisture	9.17	8.93	9.20
Total nitrogen	6.74	6.50	6.41
Uric acid nitrogen	3.80	3.82	3.77
Ammonia nitrogen	.74	.66	.69
Ether extract	2.77	3.19	3.25
Crude fiber	8.60	8.79	8.47
Nitrogen free extract	45.22	42.61	45.54
Uric acid	11.38	11.46	11.29
Ammonia	.90	.81	.85
Ash	8.24	11.59	9.25
Crude protein	13.71	12.62	12.15

	Excrement A-7	Excrement A-8	Excrement A-9
Moisture	10.63	9.85	9.83
Total nitrogen	6.01	6.02	6.03
Uric acid nitrogen	3.80	3.84	3.80
Ammonia nitrogen	.66	.71	.70
Ether extract	2.88	2.76	2.81
Crude fiber	8.11	8.45	8.81
Nitrogen free extract	46.81	47.42	47.57
Uric acid	11.40	11.50	11.38
Ammonia	.81	.87	.84
Ash	9.74	10.00	9.20
Crude protein	9.62	9.15	9.56

EXCREMENT ANALYSIS—*Continued.*

	Excrement B-1	Excrement B-2	Excrement B-3
Moisture	4.91	6.52	3.59
Total nitrogen	6.10	6.43	6.18
Uric acid nitrogen	3.33	3.13	3.15
Ammonia nitrogen	.24	.36	.44
Ether extract	4.15	5.34	5.45
Crude fiber	13.55	12.82	10.36
Nitrogen free extract	40.57	38.47	40.24
Uric acid	9.99	9.40	9.48
Ammonia	.29	.42	.53
Ash	10.72	8.86	14.16
Crude protein	15.81	18.17	16.19

	Excrement B-4	Excrement B-6	Excrement C-1
Moisture	5.12	5.84	6.44
Total nitrogen	6.38	6.06	6.58
Uric acid nitrogen	3.38	3.10	2.63
Ammonia nitrogen	.29	.55	.98
Ether extract	8.63	8.62	5.42
Crude fiber	13.44	10.26	10.30
Nitrogen free extract	33.27	37.58	31.36
Uric acid	10.16	9.32	7.89
Ammonia	.35	.68	1.16
Ash	12.10	12.64	18.77
Crude protein	16.93	15.06	18.65

	Excrement C-2	Excrement C-3	Excrement C-4
Moisture	8.02	7.46	8.23
Total nitrogen	5.88	5.95	6.15
Uric acid nitrogen	2.82	2.66	2.74
Ammonia nitrogen	.31	.62	.71
Ether extract	7.50	7.48	5.54
Crude fiber	12.07	9.32	11.11
Nitrogen free extract	32.55	38.50	35.10
Uric acid	8.48	7.98	8.23
Ammonia	.38	.76	.86
Ash	13.82	11.82	14.10
Crude protein	17.18	16.68	16.82

EXCREMENT ANALYSIS—*Continued.*

	Excrement C-5	Excrement C-6	Excrement D-7
Moisture	7.07	7.65	9.33
Total nitrogen	6.07	5.97	3.95
Uric acid nitrogen	2.60	2.43	2.28
Ammonia nitrogen	.46	.64	.42
Ether extract	6.40	7.55	1.99
Crude fiber	11.93	11.50	21.54
Nitrogen free extract	31.27	33.26	41.49
Uric acid	7.81	7.30	6.84
Ammonia	.56	.78	.51
Ash	16.18	13.83	10.46
Crude protein	18.77	18.12	7.84

	Excrement D-8	Excrement D-9	Excrement D-10
Moisture	9.06	6.42	6.12
Total nitrogen	4.18	4.00	3.67
Uric acid nitrogen	2.23	2.24	2.17
Ammonia nitrogen	.52	.52	.42
Ether extract	1.86	1.83	1.86
Crude fiber	26.96	29.00	27.39
Nitrogen free extract	37.70	37.50	44.06
Uric acid	6.68	6.12	6.51
Ammonia	.63	.63	.51
Ash	8.15	10.74	6.82
Crude protein	8.96	7.75	6.73

	Excrement D-11	Excrement D-12	Excrement E-2
Moisture	4.91	4.18	10.16
Total nitrogen	3.84	3.78	5.90
Uric acid nitrogen	2.12	2.05	3.33
Ammonia nitrogen	.48	.49	.58
Ether extract	2.10	2.16	3.37
Crude fiber	30.85	29.38	8.27
Nitrogen free extract	44.59	44.61	43.50
Uric acid	6.38	6.17	9.99
Ammonia	.58	.59	.70
Ash	2.63	5.19	11.58
Crude Protein	7.96	7.72	12.43

EXCREMENT ANALYSIS—*Continued.*

	Excrement E-3	Excrement E-5	Excrement E-8
Moisture	11.13	10.91	9.36
Total nitrogen	5.96	6.47	5.98
Uric acid nitrogen	2.99	3.38	3.27
Ammonia nitrogen	.72	.58	.68
Ether extract	3.96	4.91	3.88
Crude fiber	8.65	9.50	9.00
Nitrogen free extract	42.19	40.09	46.15
Uric acid	8.98	10.16	9.82
Ammonia	.87	.64	.82
Ash	10.16	7.83	8.29
Crude protein	14.06	15.96	12.68

	Excrement E-11	Excrement F-1	Excrement F-5
Moisture	8.67	10.17	9.16
Total nitrogen	6.42	9.97	9.84
Uric acid nitrogen	3.38	7.05	6.92
Ammonia nitrogen	.64	1.07	1.00
Ether extract	4.79	3.28	3.98
Crude fiber	9.03	7.39	7.88
Nitrogen free extract	41.07	26.22	27.74
Uric acid	10.16	21.16	20.76
Ammonia	.77	1.31	1.22
Ash	10.54	18.86	17.24
Crude protein	14.97	11.61	12.02

	Excrement G-1	Excrement G-2	Excrement H-1
Moisture	16.01	18.00	8.19
Total nitrogen	8.93	8.35	10.85
Uric acid nitrogen	6.60	6.21	7.44
Ammonia nitrogen	.58	.55	.76
Ether extract	2.76	2.86	2.77
Crude fiber	7.49	7.04	5.71
Nitrogen free extract	22.09	20.80	28.49
Uric acid	19.81	18.63	22.31
Ammonia	.70	.69	.92
Ash	20.17	22.03	15.00
Crude protein	10.97	9.97	16.61

Excrement Analysis—*Continued.*

	Excrement H-3	Excrement H-5	Excrement H-6
Moisture	9.31	12.18	13.13
Total nitrogen	10.00	10.36	10.37
Uric acid nitrogen	6.96	7.38	7.32
Ammonia nitrogen	.80	.96	.78
Ether extract	2.83	2.00	2.04
Crude fiber	5.88	4.05	4.18
Nitrogen free extract	30.07	20.23	20.80
Uric acid	20.87	22.13	21.96
Ammonia	.97	1.16	.94
Ash	16.05	25.60	22.71
Crude protein	14.02	12.65	14.24

	Excrement I-1	Excrement I-2	Excrement I-3
Moisture	8.61	10.89	12.11
Total nitrogen	7.95	7.63	7.17
Uric acid nitrogen	4.77	4.89	4.64
Ammonia nitrogen	.64	.50	.56
Ether extract	2.94	2.71	2.44
Crude fiber	5.92	5.42	4.94
Nitrogen free extract	35.88	33.44	30.09
Uric acid	14.31	14.67	13.93
Ammonia	.77	.60	.68
Ash	15.64	18.26	23.47
Crude Protein	15.93	14.01	12.34

	Excrement I-4	Excrement I-5	Excrement J-1
Moisture	7.29	8.01	14.12
Total nitrogen	7.41	7.25	9.60
Uric acid nitrogen	4.42	4.33	7.06
Ammonia nitrogen	.44	.46	.78
Ether extract	3.29	3.22	3.03
Crude fiber	6.53	6.53	8.48
Nitrogen free extract	41.02	41.12	20.12
Uric acid	13.26	12.99	21.18
Ammonia	.53	.56	.94
Ash	12.13	12.17	21.11
Crude protein	15.95	15.40	11.02

Excrement Analysis—*Continued.*

	Excrement J-3	Excrement J-4	Excrement J-5
Moisture	15.16	16.33	12.79
Total nitrogen	10.00	9.21	9.76
Uric acid nitrogen	7.44	6.30	7.33
Ammonia nitrogen	.72	1.00	.67
Ether extract	2.83	2.75	2.83
Crude fiber	8.47	8.43	8.40
Nitrogen free extract	20.39	19.68	20.09
Uric acid	22.33	18.91	21.98
Ammonia	.88	1.22	.82
Ash	18.44	20.72	22.06
Crude protein	11.50	11.96	11.03

	Excrement J-6	Excrement K-1	Excrement K-2
Moisture	16.39	10.12	9.87
Total nitrogen	9.90	7.15	6.96
Uric acid nitrogen	7.22	4.68	4.56
Ammonia nitrogen	.72	.70	.71
Ether extract	2.61	9.85	10.65
Crude fiber	8.19	21.97	21.48
Nitrogen free extract	18.78	21.38	21.37
Uric acid	21.67	14.03	13.96
Ammonia	.88	.84	.86
Ash	19.22	10.74	11.54
Crude protein	12.26	11.07	10.54

	Excrement K-3	Excrement K-5	Excrement K-6
Moisture	9.23	14.11	14.62
Total nitrogen	6.93	6.62	7.10
Uric acid nitrogen	4.42	4.54	4.79
Ammonia nitrogen	.67	.64	1.00
Ether extract	11.55	9.29	8.00
Crude fiber	22.35	18.21	18.20
Nitrogen free extract	21.17	17.52	17.44
Uric acid	13.26	13.61	14.36
Ammonia	.82	.78	1.22
Ash	10.00	17.46	17.83
Crude protein	11.62	9.02	8.23

EXCREMENT ANALYSIS—*Continued.*

	Excrement M-1	Excrement M-2	Excrement M-3
Moisture	11.22	9.17	10.39
Total nitrogen	8.97	9.03	9.10
Uric acid nitrogen	6.39	6.28	6.44
Ammonia nitrogen	.79	.91	.86
Ether extract	3.45	3.63	3.33
Crude fiber	8.80	9.25	8.65
Nitrogen free extract	27.96	29.35	27.50
Uric acid	19.16	18.84	19.32
Ammonia	.96	1.10	1.04
Ash	17.21	17.10	18.46
Crude protein	11.24	11.56	11.31

	Excrement M-5	Excrement M-6	Excrement N-1
Moisture	11.01	8.26	9.86
Total nitrogen	10.03	9.09	9.58
Uric acid nitrogen	7.54	6.31	6.71
Ammonia nitrogen	.72	.80	.70
Ether extract	3.45	3.74	3.86
Crude fiber	8.79	9.79	15.79
Nitrogen free extract	27.96	30.55	21.98
Uric acid	19.62	18.93	20.12
Ammonia	.87	.98	.84
Ash	17.13	15.29	13.94
Crude protein	11.17	12.46	13.61

	Excrement N-3	Excrement N-4	Excrement N-6
Moisture	10.17	11.03	10.39
Total nitrogen	9.39	9.69	9.72
Uric acid nitrogen	6.64	7.10	7.00
Ammonia nitrogen	.66	.71	.75
Ether extract	3.51	3.52	3.52
Crude fiber	14.89	15.23	14.91
Nitrogen free extract	20.57	22.84	23.16
Uric acid	19.91	21.31	21.02
Ammonia	.80	.86	.91
Ash	17.04	13.48	13.76
Crude protein	13.11	11.73	12.33

EXCREMENT ANALYSIS—*Continued.*

	Excrement O-2	Excrement O-3	Excrement O-5
Moisture	9.81	10.12	9.32
Total nitrogen	6.51	6.47	6.41
Uric acid nitrogen	4.01	3.99	4.04
Ammonia nitrogen	.76	.69	.72
Ether extract	3.81	3.88	4.01
Crude fiber	12.17	12.22	11.83
Nitrogen free extract	37.96	37.34	39.61
Uric acid	12.09	11.96	12.13
Ammonia	.92	.83	.87
Ash	12.36	12.48	11.87
Crude protein	10.88	11.17	10.36

	Excrement P-1	Excrement P-2	Excrement P-3
Moisture	5.00	6.02	5.11
Total nitrogen	6.69	6.14	6.59
Uric acid nitrogen	4.79	4.05	4.27
Ether extract	6.94	6.59	6.89
Ammonia nitrogen	.59	.61	.53
Crude fiber	12.81	10.37	12.16
Nitrogen free extract	43.93	41.61	44.32
Uric acid	14.37	12.16	12.82
Ammonia	.71	.74	.64
Ash	8.01	13.26	6.92
Crude protein	8.23	9.25	11.24

	Excrement P-4	Excrement P-5	Excrement P-6
Moisture	6.18	7.22	6.39
Total nitrogen	6.24	6.09	6.00
Uric acid nitrogen	4.01	3.94	4.03
Ammonia nitrogen	.57	.66	.51
Ether extract	7.21	6.49	6.45
Crude fiber	11.86	10.91	12.33
Nitrogen free extract	43.62	42.13	41.83
Uric acid	12.03	11.82	12.09
Ammonia	.69	.80	.62
Ash	8.01	1.130	11.12
Crude protein	10.40	9.33	9.17

EXCREMENT ANALYSIS—*Continued.*

	Excrement Q-1	Excrement Q-2	Excrement Q-3
Moisture	15.00	14.89	14.93
Total nitrogen	5.90	6.07	5.96
Uric acid nitrogen	3.77	3.84	3.72
Ammonia nitrogen	.71	.81	.76
Ether extract	3.48	3.54	3.55
Crude fiber	9.61	9.75	9.77
Nitrogen free extract	30.80	31.38	31.11
Uric acid	11.31	11.53	11.16
Ammonia	.86	.98	.92
Ash	19.94	19.06	19.25
Crude protein	9.00	8.87	9.31

	Excrement R-1	Excrement R-2	Excrement R-3
Moisture	12.13	10.39	8.96
Total nitrogen	7.95	8.22	7.98
Uric acid nitrogen	4.74	4.66	4.34
Ammonia nitrogen	.73	.68	.60
Ether extract	4.60	4.59	5.81
Crude fiber	7.21	7.61	8.07
Nitrogen free extract	26.43	29.84	35.70
Uric acid	14.22	13.98	13.01
Ammonia	.89	.83	.73
Ash	19.02	15.72	8.67
Crude protein	15.50	17.05	19.05

	Excrement R-4	Excrement R-5	Excrement
Moisture	9.17	10.16	----------
Total nitrogen	7.50	7.19	----------
Uric acid nitrogen	4.27	4.31	----------
Ammonia nitrogen	.67	.59	----------
Ether extract	5.60	5.33	----------
Crude fiber	6.55	5.93	----------
Nitrogen free extract	39.22	38.49	----------
Uric acid	12.81	12.93	----------
Ammonia	.81	.71	----------
Ash	9.83	12.14	----------
Crude protein	16.01	14.31	----------

EXCREMENT ANALYSIS—*Continued.*

	Excrement S-1	Excrement S-2	Excrement S-3
Moisture	7.92	8.36	7.26
Total nitrogen	6.63	7.40	6.47
Uric acid nitrogen	3.71	4.05	3.68
Ammonia nitrogen	.64	.69	.55
Ether extract	3.57	3.22	3.55
Crude fiber	11.31	12.24	10.36
Nitrogen free extract	42.94	39.65	42.73
Uric acid	11.14	12.16	11.03
Ammonia	.78	.84	.67
Ash	8.09	10.64	10.33
Crude Protein	14.25	12.87	14.07

	Excrement S-4	Excrement S-5	Excrement S-6
Moisture	8.03	6.96	7.06
Total nitrogen	6.55	6.74	6.68
Uric acid nitrogen	3.74	4.00	3.94
Ammonia nitrogen	.64	.56	.61
Ether extract	3.19	3.56	3.45
Crude fiber	10.64	11.66	10.99
Nitrogen free extract	43.71	40.96	41.70
Uric acid	11.22	12.01	11.83
Ammonia	.77	.68	.74
Ash	8.84	10.49	10.92
Crude protein	13.60	13.68	13.31

	Excrement T-1	Excrement T-2	Excrement T-3
Moisture	10.31	12.02	10.86
Total nitrogen	5.38	5.42	5.24
Uric acid nitrogen	3.41	3.40	3.30
Ammonia nitrogen	.77	.81	.63
Ether extract	2.51	2.46	2.59
Crude fiber	14.92	14.21	15.14
Nitrogen free extract	32.14	31.29	33.95
Uric acid	10.23	10.19	9.89
Ammonia	.94	.98	.77
Ash	21.13	21.25	19.38
Crude protein	7.55	7.60	8.19

EXCREMENT ANALYSIS—*Continued.*

	Excrement T-4	Excrement T-5	Excrement U-1
Moisture	11.12	10.99	8.91
Total nitrogen	5.23	5.33	6.60
Uric acid nitrogen	3.29	3.30	3.94
Ammonia nitrogen	.66	.73	.80
Ether extract	2.49	2.56	3.45
Crude fiber	14.71	14.92	11.98
Nitrogen free extract	34.44	34.17	37.56
Uric acid	9.86	9.91	11.83
Ammonia	.80	.89	.97
Ash	18.53	18.45	13.63
Crude protein	8.05	8.11	11.67

	Excrement U-2	Excrement U-3	Excrement U-4
Moisture	7.93	8.62	9.33
Total nitrogen	6.47	6.25	6.27
Uric acid nitrogen	3.98	3.67	3.94
Ammonia nitrogen	.69	.78	.59
Ether extract	1.94	3.63	3.62
Crude fiber	11.75	11.36	11.17
Nitrogen free extract	39.75	39.94	40.33
Uric acid	11.90	11.02	11.82
Ammonia	.84	.95	.71
Ash	14.64	13.22	12.13
Crude protein	11.25	11.26	10.89

	Excrement U-5	Excrement V-1	Ecxrement V-2
Moisture	8.32	8.23	9.01
Total nitrogen	6.47	6.29	6.27
Uric acid nitrogen	3.97	4.00	3.92
Ammonia nitrogen	.61	.65	.73
Ether extract	3.63	3.70	3.59
Crude fiber	12.20	13.32	12.50
Nitrogen free extract	40.18	38.55	38.19
Uric acid	11.91	11.99	11.96
Ammonia	.74	.79	.89
Ash	11.26	13.15	13.90
Crude protein	11.76	10.27	10.16

EXCREMENT ANALYSIS—*Continued.*

	Excrement W-1	Excrement W-2	Excrement W-3
Moisture	6.93	7.06	6.89
Total nitrogen	6.41	6.51	6.46
Uric acid nitrogen	4.35	4.39	4.42
Ammonia nitrogen	.52	.63	.48
Ether extract	3.79	3.53	3.52
Crude fiber	14.17	14.33	14.56
Nitrogen free extract	42.29	40.94	41.67
Uric acid	12.06	13.16	13.27
Ammonia	.63	.77	.58
Ash	9.47	10.80	9.66
Crude protein	9.66	9.41	9.85

DIGESTIVE COEFFICIENTS

CHICKEN A-1

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	82.86	47.56	35.30	42.60
Crude protein	14.33	7.26	7.07	49.33
Ether extract	3.94	1.99	1.95	49.49
Crude fiber	6.00	5.45	0.55	9.10
Nitrogen free extract	58.59	32.86	25.73	43.91

CHICKEN A-2

Organic matter	82.86	47.74	35.12	42.38
Crude protein	14.33	7.77	6.56	45.89
Ether extract	3.94	1.89	2.05	52.03
Crude fiber	6.00	5.34	0.66	11.01
Nitrogen free extract	58.59	32.74	25.85	44.12

CHICKEN A-3

Organic matter	75.95	43.28	32.67	43.01
Crude protein	13.13	7.15	5.98	45.54
Ether extract	3.61	1.70	1.91	52.91
Crude fiber	5.50	5.01	0.49	8.97
Nitrogen free extract	53.71	29.42	24.29	45.22

CHICKEN A-4

Organic matter	90.63	49.20	41.43	45.70
Crude protein	15.67	9.60	6.07	38.73
Ether extract	4.31	1.93	2.38	52.22
Crude fiber	6.56	6.02	0.54	8.16
Nitrogen free extract	64.09	31.65	32.44	56.01

CHICKEN A-5

Organic matter	98.40	50.06	48.34	49.12
Crude protein	17.02	9.40	7.62	44.77
Ether extract	4.68	2.37	2.31	49.35
Crude fiber	7.12	6.55	0.57	7.99
Nitrogen free extract	69.58	31.74	37.84	54.38

CHICKEN A-6

Organic matter	98.40	53.94	44.46	45.19
Crude protein	17.02	9.48	7.54	44.59
Ether extract	4.68	2.53	2.15	45.94
Crude fiber	7.12	6.41	.71	10.00
Nitrogen free extract	69.58	35.52	34.06	48.91

DIGESTIVE COEFFICIENTS—*Continued.*

CHICKEN A-7

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	207.18	102.79	104.39	50.38
Crude protein	35.83	14.48	21.35	59.92
Ether extract	9.86	4.32	5.54	56.91
Crude fiber	15.00	13.78	1.22	8.19
Nitrogen free extract	146.49	70.21	76.28	52.07

CHICKEN A-8

Organic matter	207.18	100.43	106.75	51.52
Crude protein	35.83	13.36	22.47	62.71
Ether extract	9.86	4.03	5.83	59.12
Crude fiber	15.00	13.80	1.20	8.00
Nitrogen free extract	146.49	69.24	77.25	52.73

CHICKEN A-9

Organic matter	177.01	90.75	86.26	49.85
Crude protein	30.65	12.61	18.04	58.89
Ether extract	8.42	3.71	4.71	55.92
Crude fiber	12.81	11.64	1.17	9.13
Nitrogen free extract	125.13	62.79	62.34	49.82

TOTALS AND AVERAGES OF CHICKENS A-1, -2, -3, -4, -5, -6, -7, -8, and -9

	Total grams Intake	Total grams Outgo	Total grams Assimilated	Coefficient
Organic matter	1120.47	585.75	534.72	47.72
Crude protein	193.81	91.11	102.70	52.91
Ether extract	53.30	24.47	28.83	54.09
Crude fiber	81.11	74.00	7.11	8.76
Nitrogen free extract	792.25	396.17	396.08	49.99

CHICKEN B-1

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	140.96	21.48	119.48	84.76
Crude protein	16.48	4.58	11.90	72.20
Ether extract	8.16	1.21	6.95	85.17
Crude fiber	4.16	3.93	0.23	5.62
Nitrogen free extract	112.16	11.76	100.40	89.51

DIGESTIVE COEFFICIENTS—*Continued.*

CHICKEN B-2

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	140.96	22.72	118.24	83.88
Crude protein	16.48	5.45	11.01	66.49
Ether extract	8.16	1.63	6.53	80.02
Crude fiber	4.16	3.91	0.25	6.12
Nitrogen free extract	112.16	11.73	100.43	89.54

CHICKEN B-3*

Organic matter	70.48	13.73	56.75	80.52
Crude protein	8.24	3.08	5.16	62.63
Ether extract	4.08	1.03	3.05	74.75
Crude fiber	2.08	1.97	0.11	5.37
Nitrogen free extract	6.08	7.65	48.43	86.62

* Only two days completed on digestion period—went off feed.

CHICKEN B-4

Organic matter	140.96	21.53	119.43	84.01
Crude protein	16.48	5.49	10.99	66.08
Ether extract	8.16	2.50	5.66	69.36
Nitrogen free extract	112.16	9.64	102.52	91.55
Crude fiber	4.16	3.90	0.26	6.31

CHICKEN B-6

Organic matter	138.54	22.15	116.39	84.01
Crude protein	16.96	4.51	11.45	71.74
Ether extract	7.90	2.58	5.32	67.00
Crude fiber	4.03	3.79	0.24	5.98
Nitrogen free extract	110.65	11.27	99.38	89.81

TOTALS AND AVERAGES OF CHICKENS B-1, -2, -3, -4, and -6

	Total grams Intake	Total grams Outgo	Total grams Assimilated	Coefficient
Organic matter	631.90	101.61	530.29	83.91
Crude protein	73.64	23.11	50.53	68.71
Ether extract	36.46	8.95	27.51	75.26
Crude fiber	18.59	17.50	1.09	5.86
Nitrogen free extract	503.21	52.05	451.16	89.40

DIGESTIVE COEFFICIENTS—*Continued.*

CHICKEN C-2

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	132.10	28.07	104.03	78.71
Crude protein	18.75	6.96	11.79	62.88
Ether extract	4.35	3.03	1.22	28.27
Crude fiber	4.90	4.88	0.02	0.40
Nitrogen free extract	104.10	13.18	90.92	87.33

CHICKEN C-1

Organic matter	140.64	26.96	112.40	79.92
Crude protein	20.00	7.48	12.52	62.10
Ether extract	4.64	2.20	2.44	52.58
Crude fiber	4.96	4.22	.74	15.14
Nitrogen free extract	111.04	12.86	98.18	88.41

CHICKEN C-3

Organic matter	140.64	33.45	107.17	76.20
Crude protein	20.00	7.75	12.25	61.12
Ether extract	4.64	3.47	1.17	25.21
Crude fiber	4.96	4.33	0.63	12.17
Nitrogen free extract	111.04	17.90	93.14	83.88

CHICKEN C-4

Organic matter	127.80	28.35	99.34	77.66
Crude protein	18.12	6.98	11.14	61.47
Ether extract	4.20	2.20	2.00	47.61
Crude fiber	4.85	4.61	0.24	4.94
Nitrogen free extract	100.63	14.56	86.07	85.43

CHICKEN C-5

Organic matter	140.64	27.33	113.39	80.55
Crude protein	20.00	7.50	12.50	62.50
Ether extract	4.64	2.56	2.08	44.87
Crude fiber	4.96	4.77	0.19	3.83
Nitrogen free extract	114.04	12.50	98.54	88.84

CHICKEN C-6

Organic matter	140.64	30.80	109.65	77.96
Crude protein	20.00	7.97	12.03	60.15
Ether extract	4.64	3.30	1.30	28.01
Crude fiber	4.96	4.90	0.06	1.41
Nitrogen free extract	111.04	14.63	96.41	86.82

DIGESTIVE COEFFICIENTS—*Continued.*

TOTALS AND AVERAGES OF CHICKENS C-1, -2, -3, -4, -5, and -6

	Total grams Intake	Total grams Outgo	Total grams Assimilated	Coefficient
Organic matter	822.46	174.74	647.72	78.50
Crude protein	116.87	44.64	72.23	61.70
Ether extract	27.11	16.76	10.35	37.76
Crude fiber	29.59	27.71	1.88	5.40
Nitrogen free extract	648.89	85.03	563.26	86.78

CHICKEN D-7

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	206.06	78.66	127.40	61.82
Crude protein	27.88	8.46	19.42	69.69
Ether extract	7.24	2.14	5.10	70.44
Crude fiber	28.99	23.26	5.73	19.76
Nitrogen free extract	141.93	44.80	97.13	68.43

CHICKEN D-8

Organic matter	206.06	72.22	133.84	64.94
Crude protein	27.88	8.70	19.18	68.79
Ether extract	7.24	1.80	5.44	75.13
Crude fiber	28.99	25.16	3.83	13.21
Nitrogen free extract	141.93	36.56	105.37	74.24

CHICKEN D-9

Organic matter	206.06	70.75	135.28	65.65
Crude protein	27.88	7.20	20.68	74.17
Ether extract	7.24	1.70	5.54	76.51
Crude fiber	28.99	26.97	2.01	6.93
Nitrogen free extract	141.93	34.88	107.05	75.43

CHICKEN D-10

Organic matter	206.06	73.65	132.39	64.73
Crude protein	27.88	6.20	21.68	77.79
Ether extract	7.24	1.72	5.52	76.24
Crude fiber	28.99	25.20	3.79	13.07
Nitrogen free extract	141.93	40.53	101.40	71.44

DIGESTIVE COEFFICIENTS—*Continued.*

CHICKEN D-11

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	206.06	7[illegible].85	127.21	61.73
Crude protein	27.88	6.93	20.95	75.14
Ether extract	7.24	1.83	5.41	74.72
Crude fiber	28.99	26.84	2.15	7.41
Nitrogen free extract	141.93	43.25	98.68	69.52

CHICKEN D-12

Organic matter	206.06	74.65	131.41	63.82
Crude protein	27.88	6.87	21.01	75.35
Ether extract	7.24	1.93	5.31	73.48
Crude fiber	28.99	26.15	2.84	9.79
Nitrogen free extract	141.93	39.70	102.23	72.02

TOTAL AND AVERAGES OF CHICKENS D-7, -8, -9, 10, -11, and -12

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	1236.36	448.78	787.53	63.78
Crude protein	167.28	44.36	122.92	73.49
Ether extract	43.44	11.12	32.32	74.42
Crude fiber	173.94	153.58	20.36	11.69
Nitrogen free extract	851.58	239.72	611.86	88.18

CHICKEN E-2

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	218.38	36.49	181.89	83.29
Crude protein	25.75	6.71	19.04	73.94
Ether extract	14.40	1.82	12.58	87.43
Crude fiber	4.56	4.47	0.09	1.97
Nitrogen free extract	173.65	23.49	150.16	86.47

CHICKEN E-3

Organic matter	218.38	34.78	183.60	84.07
Crude protein	25.75	7.10	18.65	72.42
Ether extract	14.40	2.00	12.40	86.16
Crude fiber	4.56	4.37	0.19	4.16
Nitrogen free extract	173.65	21.31	152.34	87.72

DIGESTIVE COEFFICIENTS—*Continued.*

CHICKEN E-5

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	218.38	29.13	189.25	86.65
Crude protein	25.75	6.62	19.13	74.29
Ether extract	14.40	2.03	12.37	85.96
Crude fiber	4.56	3.94	0.62	13.59
Nitrogen free extract	173.65	16.54	157.11	90.47

CHICKEN E-8

Organic matter	202.11	32.27	169.84	84.03
Crude protein	23.83	5.70	18.13	76.08
Ether extract	13.33	1.75	11.58	86.93
Crude fiber	4.22	4.05	0.17	4.02
Nitrogen free extract	160.71	20.77	139.94	87.07

CHICKEN E-11

Organic matter	205.53	30.03	175.50	85.34
Crude protein	24.24	6.43	17.81	73.47
Ether extract	13.56	2.06	11.50	84.86
Nitrogen free extract	163.44	17.66	145.78	89.19

TOTAL AND AVERAGES OF CHICKENS E-2, -3, -5, -8, and -11

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	1062.78	162.70	900.08	84.67
Crude protein	125.32	32.56	92.76	74.04
Ether extract	70.09	9.66	60.43	86.26
Crude fiber	22.19	20.71	1.48	6.66
Nitrogen free extract	845.10	99.77	745.33	88.18

CHICKEN F-1. RATION: CORN MEAL 2-3, MEAT SCRAP AND BONE MEAL 1-3.

	Gr. Corn meal Intake	Gr. Meat Scrap Intake	Total Intake	Total Outgo	Total Assimilation	Gr. Corn meal Digested	Gr. Meat Scrap Digested	Coefficient Meat Scraps
Organic matter	137.02	52.36	189.38	27.91	161.47	116.01	45.46	86.82
Crude protein	16.16	30.44	46.60	6.68	39.92	11.96	27.96	91.86
Ether extract	9.04	11.10	20.14	1.88	18.26	7.79	10.47	94.31
Crude fiber	2.86	1.64	4.50	4.25	0.25	0.19	0.06	3.46
Nitrogen free extract	108.96	9.17	118.13	15.08	103.05	96.08	6.97	76.00

CHICKEN F-5.

Organic matter	137.02	52.36	189.38	27.91	161.47	116.01	45.46	86.82
Crude protein	16.16	30.44	46.60	6.49	40.11	11.96	28.15	92.49
Ether extract	9.04	11.10	20.14	2.15	17.99	7.79	10.20	91.96
Crude fiber	2.86	1.64	4.50	4.26	0.239	0.19	0.049	3.01
Nitrogen free extract	108.96	9.17	118.13	14.98	103.15	96.08	7.07	77.13

TOTAL AND AVERAGES OF CHICKENS F-1 AND F-5

BEEF SCRAP AND BONE MEAL ONLY

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	104.72	13.80	90.92	86.82
Crude protein	60.88	4.77	56.11	92.17
Ether extract	22.20	1.53	20.67	93.13
Crude fiber	3.28	3.171	0.109	3.23
Nitrogen free extract	18.34	4.30	14.04	76.56

CHICKEN G-1 RATION: CORN MEAL 80%, TANKAGE 20%.

	Gr. Corn meal Intake	Gr. Tankage Intake	Total Intake	Total Outgo	Total Assimilation	Gr. Corn meal Digested	Gr. Tankage Digested	Coefficient Tankage
Organic matter	164.42	38.20	202.62	30.75	171.87	139.21	32.66	85.49
Crude protein	19.39	27.93	47.32	7.79	39.56	14.36	25.20	90.26
Ether extract	10.84	6.77	17.61	1.96	15.65	9.35	6.30	93.17
Crude fiber	3.43	2.20	5.63	5.32	0.32	0.23	0.09	4.00
Nitrogen free extract	130.75	1.30	132.05	15.69	116.36	115.29	1.07	82.00

CHICKEN G-2.

Organic matter	143.87	33.43	177.30	26.87	150.43	121.81	28.62	85.61
Crude protein	16.96	24.44	41.40	6.58	34.82	12.55	22.27	91.14
Ether extract	9.49	5.92	15.41	1.89	13.52	8.18	5.34	90.28
Crude fiber	3.00	1.93	4.93	4.65	0.28	0.20	0.08	3.88
Nitrogen free extract	114.40	1.13	115.53	13.73	101.80	100.87	0.93	81.76

TOTAL AND AVERAGES OF CHICKENS G-1 AND -2.

TANKAGE ONLY

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	71.63	10.35	61.28	85.50
Crude protein	52.37	4.90	47.47	90.70
Ether extract	12.69	1.05	11.64	91.71
Crude fiber	4.13	3.96	0.17	3.94
Nitrogen free extract	2.43	0.43	2.00	81.88

CHICKEN H-1. RATION: CORN MEAL 80%, BLOOD MEAL 20%.

	Gr. Corn Meal Intake	Gr. Blood Meal Intake	Total Intake	Total Outgo	Grams Assimilation	Gr. Corn Meal Digested	Gr. Blood Meal Digested	Coefficient Blood Meal
Organic matter	143.87	36.62	180.49	26.27	154.22	121.81	32.41	88.50
Crude protein	16.96	34.90	51.86	8.14	43.72	12.55	31.17	89.32
Ether extract	9.49	0.24	9.73	1.36	8.37	8.18	0.19	79.16
Crude fiber	3.00	0.00	3.00	2.80	0.20	0.20	----------	----------
Nitrogen free extract	114.40	1.47	115.87	13.95	101.92	100.89	1.05	71.33

CHICKEN H-3.

	Gr. Corn Meal Intake	Gr. Blood Meal Intake	Total Intake	Total Outgo	Grams Assimilation	Gr. Corn Meal Digested	Gr. Blood Meal Digested	Coefficient Blood Meal
Organic matter	132.32	20.92	144.24	21.66	122.58	104.41	18.17	86.85
Crude protein	14.54	19.94	34.48	5.75	28.73	10.76	17.97	90.17
Ether extract	8.13	0.14	8.27	1.16	7.11	7.01	0.11	77.31
Crude fiber	2.58	----------	2.58	2.41	0.17	0.17	----------	----------
Nitrogen free extract	98.06	.84	98.90	12.33	86.57	85.93	0.61	73.14

CHICKEN H-5.

	Gr. Corn Meal Intake	Gr. Blood Meal Intake	Total Intake	Total Outgo	Grams Assimilation	Gr. Corn Meal Digested	Gr. Blood Meal Digested	Coefficient Blood Meal
Organic matter	143.87	36.62	180.49	26.88	153.61	121.81	31.80	86.83
Crude protein	16.96	34.90	51.86	8.73	43.13	12.55	30.55	87.63
Ether extract	9.49	0.24	9.73	1.38	8.37	8.18	0.19	80.45
Crude fiber	3.00	----------	3.00	2.80	.20	.20	----------	----------
Nitrogen free extract	114.40	1.47	115.87	13.96	101.91	100.88	1.03	70.18

CHICKEN H-6. RATION: CORN MEAL 80%, BLOOD MEAL 20%.

	Gr. Corn Meal Intake	Gr. Blood Meal Intake	Total Intake	Total Outgo	Grams Assimilation	Gr. Corn Meal Digested	Gr. Blood Meal Digested	Coefficient Blood Meal
Organic matter	143.87	36.62	180.49	27.68	152.81	121.81	31.00	84.65
Crude protein	16.96	34.90	51.86	9.54	42.32	12.55	29.77	85.31
Ether extract	9.49	0.24	9.73	1.37	8.36	8.18	0.18	76.46
Crude fiber	3.00	----------	3.00	2.80	0.20	0.20	----------	----------
Nitrogen free extract	114.40	1.47	115.87	13.94	101.93	100.88	1.05	71.56

TOTAL AND AVERAGES OF CHICKENS H-1, -3, -5, AND -6

BLOOD MEAL ONLY

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	130.78	17.40	113.38	86.71
Crude protein	124.64	15.15	109.49	88.10
Ether extract	0.86	.19	.67	78.36
Nitrogen free extract	5.25	2.02	3.23	71.55

CHICKEN I-1. RATION: CORN MEAL 80%, DRIED BUTTERMILK 20%.

	Gr. Corn Meal Intake	Gr. Buttermilk Intake	Total Intake	Total Outgo	Grams Assimilation	Gr. Corn Meal Digested	Gr. Buttermilk Digested	Coefficient Buttermilk
Organic matter	164.42	40.06	204.40	32.72	171.66	139.21	32.45	81.00
Crude protein	19.39	16.39	35.78	8.61	27.17	14.36	12.81	78.19
Ether extract	10.84	0.475	11.32	1.59	9.73	9.35	.38	79.10
Crude fiber	3.43		3.43	3.20	0.23	0.23		
Nitrogen free extract	130.75	23.18	153.93	19.38	134.55	115.29	19.26	83.11

CHICKEN I-2.

	Gr. Corn Meal Intake	Gr. Buttermilk Intake	Total Intake	Total Outgo	Grams Assimilation	Gr. Corn Meal Digested	Gr. Buttermilk Digested	Coefficient Buttermilk
Organic matter	164.42	40.06	204.48	32.84	171.64	139.21	32.43	80.92
Crude protein	19.39	16.39	35.78	8.27	27.51	14.36	13.15	80.23
Ether extract	10.84	0.475	11.32	1.60	9.72	9.35	0.37	77.19
Crude fiber	3.43	0.00	3.45	3.20	0.28	0.23		
Nitrogen free extract	130.75	23.18	153.93	19.73	134.20	115.29	18.91	81.62

CHICKEN I-3.

	Gr. Corn Meal Intake	Gr. Buttermilk Intake	Total Intake	Total Outgo	Grams Assimilation	Gr. Corn Meal Digested	Gr. Buttermilk Digested	Coefficient Buttermilk
Organic matter	80.84	19.69	100.53	15.94	84.59	68.45	16.14	81.98
Crude protein	9.53	8.06	17.59	3.95	13.64	7.06	6.58	81.67
Ether extract	5.33	[0.234	5.56	0.78	4.78	4.59	.19	80.46
Crude fiber	1.69		1.69	1.58	0.11	0.11		
Nitrogen free extract	64.29	11.40	75.69	9.63	66.06	56.69	9.37	82.16

CHICKEN I-4. RATION: CORN MEAL 80%, DRIED BUTTERMILK 20%.

	Gr. Corn Meal Intake	Gr. Buttermilk Intake	Total Intake	Total Outgo	Grams Assimilation	Gr. Corn Meal Digested	Gr. Buttermilk Digested	Coefficient Buttermilk
Organic matter	164.42	40.06	204.48	32.77	171.71	139.21	32.50	80.00
Crude protein	19.39	16.39	35.78	7.82	27.96	14.36	13.60	83.02
Ether extract	10.84	0.475	11.32	1.61	9.71	9.35	0.36	76.19
Crude fiber	3.43		3.43	3.20	.23	0.23		
Nitrogen free extract	130.75	23.18	153.93	20.10	133.83	115.29	18.54	80.00

CHICKEN I-5.

	Gr. Corn Meal Intake	Gr. Buttermilk Intake	Total Intake	Total Outgo	Grams Assimilation	Gr. Corn Meal Digested	Gr. Buttermilk Digested	Coefficient Buttermilk
Organic matter	164.42	40.06	204.48	32.53	171.95	139.21	32.74	80.06
Crude protein	19.39	16.39	35.78	7.55	28.23	14.33	13.87	84.67
Ether extract	10.84	0.475	11.32	1.59	9.73	9.35	.38	80.13
Crude fiber	3.43		3.43	3.20	.23	0.23		
Nitrogen free extract	130.75	23.18	153.93	20.15	133.78	115.29	18.49	78.81

TOTAL AND AVERAGES OF CHICKENS I-1, -2, -3, -4, AND 5

DRIED BUTTERMILK ONLY

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	179.93	33.67	146.26	80.79
Crude protein	73.62	13.61	60.01	81.55
Ether extract	2.134	.454	1.68	78.01
Crude fiber				
Nitrogen free extract	104.12	22.98	84.57	81.14

CHICKEN J-1. RATION: CORN MEAL 2-3, SOYBEAN MEAL 1-3.

	Gr. Corn Meal Intake	Gr. Soybean Meal Intake	Total Intake	Total Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Soybean Meal Digested	Coefficient Soybean Meal
Organic matter	137.02	67.61	204.63	35.76	168.97	116.01	52.96	78.33
Crude protein	16.16	33.68	49.84	9.26	40.63	11.96	28.67	85.13
Ether extract	9.04	6.08	15.12	2.61	12.61	7.79	4.82	79.32
Crude fiber	2.86	4.56	7.42	7.13	0.29	0.19	0.10	2.16
Nitrogen free extract	108.96	23.30	132.26	16.81	115.45	96.08	19.37	83.16

CHICKEN J-3.

	Gr. Corn Meal Intake	Gr. Soybean Meal Intake	Total Intake	Total Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Soybean Meal Digested	Coefficient Soybean Meal
Organic matter	137.02	67.61	204.63	36.29	168.34	116.01	52.33	77.39
Crude protein	16.16	33.68	49.84	9.66	40.18	11.96	28.22	83.79
Ether extract	9.04	6.08	15.12	2.38	12.74	7.79	4.95	81.46
Crude fiber	2.86	4.56	7.42	7.12	0.30	0.19	0.11	2.48
Nitrogen free extract	108.96	23.30	132.26	17.13	115.13	96.08	19.05	81.78

CHICKEN J-4.

	Gr. Corn Meal Intake	Gr. Soybean Meal Intake	Total Intake	Total Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Soybean Meal Digested	Coefficient Soybean Meal
Organic matter	96.94	47.83	144.77	25.76	119.01	82.02	36.99	77.33
Crude protein	11.43	23.82	35.25	7.18	28.07	8.46	19.61	82.34
Ether extract	6.39	4.30	10.69	1.65	9.04	5.51	3.53	82.16
Crude fiber	2.03	3.23	5.26	5.06	0.20	.13	0.07	1.98
Nitrogen free extract	77.09	16.48	93.57	11.81	81.76	67.98	13.78	83.62

CHICKEN J-5. RATION: CORN MEAL 2-3, SOYBEAN MEAL 1-3.

	Gr. Corn Meal Intake	Gr. Soybean Meal Intake	Total Intake	Total Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Soybean Meal Digested	Coefficient Soybean Meal
Organic matter	137.02	67.61	204.63	36.01	168.62	116.01	52.61	77.81
Crude protein	16.16	33.68	49.84	9.38	40.46	11.96	28.50	84.63
Ether extract	9.04	6.08	15.12	2.41	12.71	7.79	4.92	80.97
Crude fiber	2.86	4.56	7.42	7.14	0.28	0.10	0.09	2.00
Nitrogen free extract	108.96	23.30	132.26	17.08	115.18	96.08	19.10	82.00

CHICKEN J-6.

Organic matter	137.02	67.61	204.63	36.41	168.22	116.01	52.21	77.22
Crude protein	16.16	33.68	49.84	10.67	39.17	11.96	27.21	80.79
Ether extract	9.04	6.08	15.12	2.27	12.85	7.79	5.06	83.15
Crude fiber	2.86	4.56	7.42	7.13	0.29	0.19	0.10	2.19
Nitrogen free extract	108.96	23.30	132.26	16.34	115.92	96.08	19.84	85.17

TOTAL AND AVERAGES OF CHICKENS J-1, -3, -4, -5, AND -6.

SOYBEAN MEAL ONLY

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	318.27	71.17	247.10	77.61
Crude protein	158.54	26.33	132.21	83.33
Ether extract	28.62	5.34	23.28	81.41
Crude fiber	21.47	21.00	0.47	2.16
Nitrogen free extract	109.68	26.54	91.40	83.14

Chicken K-1. Ration: Corn Meal 2-3, Peanut Meal (Fat not Extracted) 1-3.

	Gr. Corn Meal Intake	Gr. Peanut Meal Intake	Total Intake	Total Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Peanut Meal Digested	Coefficient Peanut Meal
Organic matter	137.02	71.20	208.22	45.01	163.21	116.01	47.20	66.29
Crude protein	16.16	17.04	33.20	7.75	25.45	11.96	13.49	79.18
Ether extract	9.04	28.48	37.52	6.90	30.62	7.79	22.83	80.19
Crude fiber	2.86	13.28	16.14	15.38	0.76	0.19	0.57	4.36
Nitrogen free extract	108.96	12.40	121.36	14.97	106.39	96.08	10.31	83.16
Chicken K-2.								
Organic matter	137.02	71.20	208.22	45.10	163.12	116.01	47.11	66.16
Crude protein	16.16	17.04	33.20	7.38	25.82	11.96	13.96	81.36
Ether extract	9.04	28.48	37.52	7.46	30.06	7.79	22.27	78.21
Crude fiber	2.86	13.28	16.14	15.29	0.85	0.19	0.66	5.00
Nitrogen free extract	108.96	12.40	121.36	14.96	106.40	96.08	10.32	83.28
Chicken K-3.								
Organic matter	137.02	71.20	208.22	46.03	162.19	116.01	46.18	64.87
Crude protein	16.16	17.04	33.20	8.02	25.18	11.96	13.22	77.63
Ether extract	9.04	28.48	37.52	7.97	29.55	7.79	21.76	76.41
Crude fiber	2.86	13.28	16.14	15.42	0.72	0.19	0.53	3.99
Nitrogen free extract	168.96	12.40	121.36	14.61	106.75	96.08	10.67	86.12

CHICKEN K-5. RATION: CORN MEAL 2-3, PEANUT MEAL (FAT NOT EXTRACTED) 1-3.

	Gr. Corn Meal Intake	Gr. Peanut Meal Intake	Total Intake	Total Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Peanut Meal Digested	Coefficient Peanut Meal
Organic matter	137.02	71.20	208.22	45.42	162.80	116.01	46.79	65.71
Crude protein	16.16	17.04	33.20	7.58	25.62	11.96	13.66	80.19
Ether extract	9.04	28.48	37.52	7.81	29.71	7.79	21.92	76.98
Crude fiber	2.86	13.28	16.14	15.30	0.84	0.19	0.65	4.89
Nitrogen free extract	108.96	12.40	121.36	14.72	106.64	96.08	10.56	85.17

CHICKEN K-6.

Organic matter	137.02	71.20	208.22	44.63	163.59	116.01	47.58	66.69
Crude protein	16.16	17.04	32.20	7.08	26.12	11.96	14.16	83.14
Ether extract	9.04	28.48	37.52	6.88	30.64	7.79	22.85	80.26
Crude fiber	2.86	13.28	16.14	15.66	0.48	0.19	0.29	2.16
Nitrogen free extract	108.96	12.40	121.36	15.00	106.36	96.08	10.28	82.98

TOTAL AND AVERAGES OF CHICKENS K-1, -2, -3, -5, AND -6.

PEANUT MEAL ONLY

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	356.00	121.14	234.86	65.94
Crude protein	85.20	16.81	68.39	80.30
Ether extract	142.40	30.77	111.63	78.41
Crude fiber	66.40	63.70	2.70	4.08
Nitrogen free extract	62.00	9.86	52.14	84.14

CHICKEN M-1. RATION: CORN MEAL 80%, FISH MEAL 20%.

	Gr. Corn Meal Intake	Gr. Fish Meal Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Fish Meal Digested	Coefficient
Organic matter	165.45	28.18	193.63	30.11	163.52	137.69	25.83	92.01
Crude protein	17.33	23.47	40.80	6.58	34.22	12.74	21.48	91.50
Ether extract	10.66	4.71	15.37	2.02	13.35	9.00	4.35	92.30
Crude fiber	5.52		5.52	5.15	0.37	0.37		
Nitrogen free extract	131.94		131.94	16.36	115.58	115.58		

CHICKEN M-2.

	Gr. Corn Meal Intake	Gr. Fish Meal Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Fish Meal Digested	Coefficient
Organic matter	163.35	27.82	191.17	29.60	161.57	135.94	25.63	92.13
Crude protein	17.10	23.17	40.27	6.36	33.91	12.56	21.35	92.16
Ether extract	10.52	4.65	15.17	2.00	13.17	8.89	4.28	91.98
Crude fiber	5.45		5.45	5.09	0.36	0.36		
Nitrogen free extract	130.28		130.28	16.15	114.13	114.13		

CHICKEN M-3.

	Gr. Corn Meal Intake	Gr. Fish Meal Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Fish Meal Digested	Coefficient
Organic matter	166.85	28.41	195.26	30.48	164.78	138.86	25.92	91.23
Crude protein	17.47	23.66	41.13	6.79	34.34	12.84	21.50	90.89
Ether extract	10.75	4.75	15.50	2.00	13.50	9.08	4.42	93.00
Crude fiber	5.57		5.57	5.19	0.38	0.38		
Nitrogen free extract	133.06		133.06	16.50	116.56	116.56		

CHICKEN M-5. RATION: CORN MEAL 80%. FISH MEAL 20%

	Gr. Corn Meal Intake	Gr. Fish Meal Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Fish Meal Digested	Coefficient Fish Meal
Organic matter	166.85	28.41	195.26	30.37	164.89	138.86	26.03	91.62
Crude protein	17.47	23.66	41.13	6.59	34.54	12.84	21.70	91.73
Ether extract	10.75	4.75	15.50	2.09	13.41	9.08	4.33	91.14
Crude fiber	5.57		5.57	5.19	0.38	0.38		
Nitrogen free extract	133.06		133.06	16.50	116.56	116.56		

CHICKEN M-6.

	Gr. Corn Meal Intake	Gr. Fish Meal Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Fish Meal Digested	Coefficient Fish Meal
Organic matter	166.85	28.41	195.26	30.44	164.82	138.86	25.96	91.38
Crude protein	17.47	23.66	41.13	6.73	34.40	12.84	21.56	91.12
Ether extract	10.75	4.75	15.50	2.02	13.48	9.08	4.40	92.71
Crude fiber	5.57		5.57	5.19	0.38	0.38		
Nitrogen free extract	133.06		133.06	16.50	115.56	116.56		

TOTAL AND AVERAGES OF CHICKENS M-1, -2, -3, -5, AND -6.

FISH MEAL ONLY

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	141.23	11.86	129.37	91.60
Crude protein	117.62	10.03	107.59	91.48
Ether extract	23.61	1.86	21.75	92.24
Crude fiber				
Nitrogen free extract				

CHICKEN N-1. RATION: CORN MEAL 2-3. COTTONSEED MEAL 1-3.

	Gr. Corn Meal Intake	Gr. Cottonseed Meal Intake	Total Intake grams	Total Outgo grams	Total Assimilation grams	Gr. Corn Meal Digested	Gr. Cottonseed Meal Digested	Coefficient Cottonseed Meal
Organic matter	139.04	67.84	206.88	42.41	164.47	115.71	48.76	71.87
Crude protein	14.56	32.24	46.80	9.94	36.86	10.70	26.16	81.13
Ether extract	8.96	6.48	15.44	2.82	12.62	7.57	5.05	78.00
Crude fiber	4.64	7.68	12.32	11.53	0.79	0.31	0.48	6.31
Nitrogen free extract	110.88	21.44	132.32	18.12	114.20	97.13	17.07	79.61

CHICKEN N-3.

	Gr. Corn Meal Intake	Gr. Cottonseed Meal Intake	Total Intake grams	Total Outgo grams	Total Assimilation grams	Gr. Corn Meal Digested	Gr. Cottonseed Meal Digested	Coefficient Cottonseed Meal
Organic matter	139.04	67.84	206.88	40.64	166.24	115.71	50.53	74.48
Crude protein	14.56	32.24	46.80	10.23	36.57	10.70	25.87	80.23
Ether extract	8.96	6.48	15.44	2.74	12.70	7.57	5.13	70.18
Crude fiber	4.64	7.68	12.32	11.62	0.70	0.31	0.39	5.13
Nitrogen free extract	110.88	21.44	132.32	16.05	116.27	97.13	19.14	89.27

CHICKEN N-4.

	Gr. Corn Meal Intake	Gr. Cottonseed Meal Intake	Total Intake grams	Total Outgo grams	Total Assimilation grams	Gr. Corn Meal Digested	Gr. Cottonseed Meal Digested	Coefficient Cottonseed Meal
Organic matter	139.04	67.84	206.88	40.54	116.34	115.71	50.63	74.63
Crude protein	14.56	32.24	46.80	8.92	37.88	10.70	27.18	84.29
Ether extract	8.96	6.48	15.44	2.68	12.76	7.57	5.19	80.11
Crude fiber	4.64	7.68	12.32	11.58	0.74	0.31	0.43	5.63
Nitrogen free extract	110.80	21.44	132.32	17.36	114.96	97.13	17.83	83.18

CHICKEN N-6. RATION: CORN MEAL 2-3. COTTONSEED MEAL 1-3.

	Gr. Corn Meal Intake	Gr. Cottonseed Meal Intake	Total Intake grams	Total Outgo grams	Total Assimilation grams	Gr. Corn Meal Digested	Gr. Cottonseed Meal Digested	Coefficient Cottonseed Meal
Organic matter	139.04	67.84	206.88	42.04	164.81	115.71	49.10	72.22
Crude protein	14.56	32.24	46.80	9.62	37.18	10.70	26.48	82.14
Ether extract	8.96	6.48	15.44	2.75	12.69	7.57	5.12	78.96
Crude fiber	4.64	7.68	12.32	11.63	0.69	0.31	0.38	4.98
Nitrogen free extract	110.88	21.44	132.32	18.07	114.25	97.13	17.12	79.84

TOTAL AND AVERAGES OF CHICKENS N-1, -3, -4, AND 6.

COTTONSEED MEAL ONLY

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	271.36	72.34	199.02	73.32
Crude protein	128.96	23.27	105.69	81.94
Ether extract	25.92	5.43	20.49	79.05
Crude fiber	30.72	29.04	1.68	5.46
Nitrogen free extract	85.76	14.60	71.16	82.92

Chicken O-2

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	208.56	34.33	174.21	83.53
Crude protein	21.84	5.77	16.07	73.60
Ether extract	13.44	1.99	11.45	85.20
Crude fiber	6.96	6.45	0.49	7.00
Nitrogen free extract	166.32	20.12	146.20	87.90

Chicken O-3

Organic matter	208.56	34.25	174.31	83.50
Crude protein	21.84	5.92	15.92	72.90
Ether extract	13.44	2.06	11.38	84.70
Crude fiber	6.96	6.48	0.48	6.90
Nitrogen free extract	166.32	19.79	146.53	88.10

Chicken O-5

Organic matter	208.56	36.21	172.35	82.60
Crude protein	21.84	5.70	16.14	73.90
Ether extract	13.44	2.21	11.23	83.60
Crude fiber	6.96	6.51	0.45	6.50
Nitrogen free extract	166.32	21.79	144.53	86.99

Totals and Averages of Chickens O-2, -3, and -5.

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	625.68	104.79	520.87	83.21
Crude protein	65.52	17.39	48.13	73.50
Ether extract	40.32	6.26	34.06	84.50
Crude fiber	20.88	19.44	1.44	6.80
Nitrogen free extract	498.96	61.70	437.26	87.60

Chicken P-1

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	207.36	88.66	118.70	57.24
Crude protein	40.56	8.40	32.16	79.28
Ether extract	12.24	7.08	5.16	42.16
Crude fiber	20.40	18.17	2.23	11.12
Nitrogen free extract	134.16	55.01	79.15	59.00

Chicken P-2

	Intake grams	Outgo grams	Assimilation grams	Coefficient grams
Organic matter	207.36	93.06	114.30	55.12
Crude protein	40.56	10.08	30.48	75.15
Ether extract	12.24	7.19	5.05	41.26
Crude fiber	20.40	18.36	2.04	10.01
Nitrogen free extract	134.16	57.43	76.73	57.19

Chicken P-3

Organic matter	207.36	92.85	114.51	55.22
Crude protein	40.56	11.36	29.20	71.98
Ether extract	12.24	6.96	5.28	43.16
Crude fiber	20.40	18.33	2.07	10.18
Nitrogen free extract	134.16	56.20	77.96	58.11

Chicken P-4

Organic matter	207.36	84.20	123.16	59.39
Crude protein	40.56	9.68	30.88	76.13
Ether extract	12.24	6.71	5.53	45.19
Crude fiber	20.40	18.33	2.07	10.14
Nitrogen free extract	134.16	49.48	84.68	63.12

Chicken P-5

Organic matter	207.36	92.94	114.42	55.17
Crude protein	40.56	10.17	30.39	74.94
Ether extract	12.24	7.08	5.16	42.16
Crude fiber	20.40	18.38	2.02	9.89
Nitrogen free extract	134.16	57.31	76.85	57.28

Chicken P-6

Organic matter	207.36	93.34	114.02	55.22
Crude protein	40.56	10.09	30.47	95.12
Ether extract	12.24	7.10	5.14	41.98
Crude fiber	20.40	18.34	2.06	10.09
Nitrogen free extract	134.16	57.71	76.35	56.91

Totals and Averages of Chickens P-1, -2, -3, -4, -5, and 6.

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	1244.16	545.05	699.11	56.19
Crude protein	243.36	59.78	183.58	75.43
Ether extract	73.44	42.12	31.32	42.64
Crude fiber	122.40	109.91	12.49	10.20
Nitrogen free extract	804.96	333.14	471.72	58.61

Chicken Q-1. Ration: Corn Meal ¾, Rye ¼.

	Gr. Corn meal Intake	Grams Rye Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn meal Digested	Grams Rye Digested	Coefficient Rye
Organic matter	136.87	45.92	182.79	32.70	150.09	113.90	36.19	78.81
Crude protein	14.33	6.24	20.57	5.58	14.99	10.53	4.46	71.49
Ether extract	8.82	1.10	9.92	2.16	7.76	7.45	0.31	27.84
Crude fiber	4.57	1.78	6.35	5.96	0.39	0.31	0.08	4.61
Nitrogen free extract	109.15	36.80	145.95	19.00	126.95	95.61	31.34	85.16
Chicken Q-2.								
Organic matter	136.87	45.92	182.79	32.98	149.81	113.90	35.91	78.20
Crude protein	14.33	6.24	20.57	5.41	15.16	10.53	4.63	74.12
Ether extract	8.82	1.10	9.92	2.16	7.76	7.45	.31	28.19
Crude fiber	4.57	1.78	6.35	5.95	0.40	0.31	.09	5.00
Nitrogen free extract	109.15	36.80	145.95	19.46	126.49	95.61	30.88	83.91
Chicken Q-3.								
Organic matter	136.87	45.92	182.79	34.10	143.78	118.90	34.88	75.74
Crude protein	14.36	6.24	20.57	5.68	14.89	10.53	4.36	69.86
Ether extract	8.82	1.10	9.92	2.17	7.75	7.45	0.30	27.14
Crude fiber	4.57	1.78	6.35	5.96	0.39	0.31	0.08	4.38
Nitrogen free extract	109.15	36.80	145.95	20.20	125.75	95.61	30.14	81.89

TOTALS AND AVERAGES OF CHICKENS Q-1, -2, AND -3.

RYE ONLY

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	137.76	30.87	106.98	77.65
Crude protein	18.72	5.27	13.45	71.85
Ether extract	3.30	2.38	0.92	27.87
Crude fiber	5.34	5.09	0.25	4.68
Nitrogen free extract	110.40	18.04	92.36	83.65

CHICKEN R-1

	Intake grams	Outgo grams	Assimilation grams	Coefficient
Organic matter	212.88	32.25	180.63	84.84
Crude protein	33.36	9.30	24.06	72.12
Ether extract	20.88	2.76	18.12	86.76
Crude fiber	4.56	4.33	0.23	5.15
Nitrogen free extract	154.08	15.86	138.22	89.71

CHICKEN R-2

Organic matter	212.88	33.68	179.20	84.13
Crude protein	33.36	9.72	23.64	70.85
Ether extract	20.88	2.61	18.27	87.49
Crude fiber	4.56	4.34	0.22	4.98
Nitrogen free extract	154.08	17.01	137.07	88.96

CHICKEN R-3

Organic matter	212.88	37.07	175.81	82.06
Crude protein	33.36	10.29	23.07	69.14
Ether extract	20.88	3.14	17.74	84.98
Crude fiber	4.56	4.36	0.20	4.37
Nitrogen free extract	154.08	19.28	134.80	87.49

CHICKEN R-4

Organic matter	212.88	44.48	168.40	79.11
Crude protein	33.36	10.57	22.79	68.31
Ether extract	20.88	3.70	17.18	82.26
Crude fiber	4.56	4.32	0.24	5.17
Nitrogen free extract	154.08	25.89	128.19	83.19

CHICKEN R-5

Organic matter	212.88	46.76	166.12	78.45
Crude protein	33.36	10.44	22.92	68.71
Ether extract	20.88	3.89	16.99	81.39
Crude fiber	4.56	4.33	0.23	4.98
Nitrogen free extract	154.08	28.10	125.98	81.76

TOTALS AND AVERAGES OF CHICKENS R-1, -2, -3, -4, AND -5.

	Total Intake grams	Total Outgo gram	Total Assimilation grams	Coefficient
Organic matter	1064.40	194.24	870.16	81.75
Crude protein	166.80	50.32	116.48	69.83
Ether extract	104.40	16.10	88.30	84.57
Crude fiber	22.80	21.68	1.12	4.91
Nitrogen free extract	770.40	106.14	664.26	86.22

Chicken S-1. Ration: Corn Meal ¾, Buckwheat ¼

	Gr. Corn meal Intake	Gr. Buckwheat Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn meal Digested	Gr. Buckwheat Digested	Coefficient Buckwheat
Organic matter	156.42	51.96	208.38	40.84	167.54	130.16	37.38	71.93
Crude protein	16.38	6.66	23.04	7.27	15.77	13.03	3.74	56.18
Ether extract	10.03	1.80	11.88	1.82	10.06	8.51	1.55	86.23
Crude fiber	5.22	5.34	10.56	9.85	.71	0.35	0.36	6.76
Nitrogen free extract	124.74	38.16	162.90	21.90	141.00	109.27	31.73	83.16
Chicken S-2.								
Organic matter	156.42	51.96	208.38	41.70	166.68	130.16	36.52	70.28
Crude protein	16.38	6.66	23.04	7.34	15.70	12.03	3.67	55.14
Ether extract	10.08	1.80	11.88	1.84	10.04	8.51	1.53	84.98
Crude fiber	5.22	5.34	10.56	9.83	0.73	0.73	0.38	7.11
Nitrogen free extract	124.74	38.16	162.90	22.69	140.21	109.27	30.94	81.07
Chicken S-3.								
Organic matter	156.42	51.96	208.38	41.76	166.62	130.16	36.46	70.15
Crude protein	16.38	6.66	23.04	7.32	15.72	12.03	3.69	55.46
Ether extract	10.08	1.80	11.88	1.85	10.03	8.51	1.52	84.19
Crude fiber	5.22	5.34	10.56	9.85	0.71	0.35	0.36	6.71
Nitrogen free extract	124.74	38.16	162.90	22.74	140.16	109.27	30.89	80.96

CHICKEN S-4. RATION: CORN MEAL ¾, BUCKWHEAT ¼.

	Gr. Corn Meal Intake	Gr. Buckwheat Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Buckwheat Digested	Coefficient Buckwheat
Organic matter	156.42	51.96	208.38	42.14	166.24	130.16	36.08	69.63
Crude protein	16.38	6.66	23.04	7.21	15.83	12.03	3.80	57.13
Ether extract	10.08	1.80	11.88	1.88	10.00	8.51	1.49	82.98
Crude fiber	5.22	5.34	10.56	9.88	0.68	0.35	0.33	6.12
Nitrogen free extract	124.74	38.16	162.90	23.17	139.73	109.27	30.46	79.81

CHICKEN S-5.

	Gr. Corn Meal Intake	Gr. Buckwheat Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Buckwheat Digested	Coefficient Buckwheat
Organic matter	156.42	51.96	208.38	41.33	167.05	130.16	36.89	70.99
Crude protein	16.38	6.66	23.04	7.25	15.79	12.03	3.76	56.39
Ether extract	10.08	1.80	11.88	1.89	9.99	8.51	1.48	82.16
Crude fiber	5.22	5.34	10.56	9.89	0.67	0.35	0.32	5.98
Nitrogen free extract	124.74	38.16	162.90	22.30	140.60	109.27	31.33	82.09

CHICKEN S-6.

	Gr. Corn Meal Intake	Gr. Buckwheat Intake	Total grams Intake	Total grams Outgo	Total Assimilation	Gr. Corn Meal Digested	Gr. Buckwheat Digested	Coefficient Buckwheat
Organic matter	156.42	51.96	208.38	42.09	166.29	130.16	36.13	69.50
Crude protein	16.38	6.66	23.04	7.32	15.72	12.03	3.69	55.38
Ether extract	10.08	1.80	11.88	1.90	9.98	8.51	1.47	81.49
Crude fiber	5.22	5.34	10.56	9.93	0.63	0.35	0.28	5.13
Nitrogen free extract	124.74	38.16	162.90	22.94	139.96	108.27	30.69	80.42

TOTALS AND AVERAGES OF CHICKENS S-1, -2, -3, -4, -5, AND -6.

BUCKWHEAT ONLY

	Total Intake grams	Total Outgo grams	Total Assimilated grams	Coefficient
Organic matter	311.76	92.30	219.46	70.39
Crude protein	39.96	17.61	22.35	55.93
Ether extract	10.80	1.76	9.04	83.73
Crude fiber	32.04	30.01	2.03	6.33
Nitrogen free extract	288.96	42.72	186.04	81.25

CHICKEN T-1. RATION: CORN MEAL ¾, RICE ¼.

	Gr. Corn Meal Intake	Gr. Rice Intake	Total grams Intake	Total grams Outgo	Total grams Assimilated	Gr. Corn Meal Digested	Gr. Rice Digested	Coefficient Rice
Organic matter	136.87	45.04	181.91	35.52	146.39	113.90	32.49	72.11
Crude protein	14.33	4.25	18.58	4.68	13.90	10.53	3.37	79.36
Ether extract	8.82	1.05	9.87	1.56	8.31	7.45	0.86	81.60
Crude fiber	4.57	5.30	9.87	9.25	0.62	0.31	0.31	5.78
Nitrogen free extract	108.15	34.44	143.59	20.03	123.56	95.61	27.95	81.16

CHICKEN T-2.

	Gr. Corn Meal Intake	Gr. Rice Intake	Total grams Intake	Total grams Outgo	Total grams Assimilated	Gr. Corn Meal Digested	Gr. Rice Digested	Coefficient Rice
Organic matter	136.87	45.04	181.91	36.12	145.79	113.90	31.89	70.80
Crude protein	14.33	4.25	18.58	4.94	13.64	10.53	3.11	73.18
Ether extract	8.82	1.05	9.87	1.60	8.27	7.45	0.82	78.47
Crude fiber	4.57	5.30	9.87	9.24	0.63	0.31	0.32	6.13
Nitrogen free extract	109.15	34.44	143.59	20.34	123.25	95.61	27.64	80.27

CHICKEN T-3.

	Gr. Corn Meal Intake	Gr. Rice Intake	Total grams Intake	Total grams Outgo	Total grams Assimilated	Gr. Corn Meal Digested	Gr. Rice Digested	Coefficient Rice
Organic matter	136.87	45.04	181.91	36.54	145.37	113.90	31.47	69.87
Crude protein	14.33	4.25	15.58	5.00	13.58	10.53	3.05	71.69
Ether extract	8.82	1.05	9.87	1.58	8.29	7.45	0.84	80.17
Crude fiber	4.57	5.30	9.87	9.25	0.62	0.31	0.31	5.96
Nitrogen free extract	109.15	34.44	143.59	20.71	122.88	95.61	27.27	79.18

CHICKEN T-4. RATION: CORN MEAL ¾, RICE ¼.

	Gr. Corn meal Intake	Gr. Rice Intake	Total grams Intake	Total grams Outgo	Total grams Assimilated	Gr. Corn meal Digested	Gr. Rice Digested	Coefficient Rice
Organic matter	136.87	45.04	178.91	37.61	144.30	113.90	30.40	67.40
Crude protein	14.33	4.25	18.58	5.07	13.51	10.53	2.98	70.09
Ether extract	8.82	1.05	9.87	1.57	8.30	7.45	0.85	80.96
Crude fiber	4.57	5.30	9.87	9.27	0.60	0.31	0.29	5.49
Nitrogen free extract	109.15	34.44	143.59	21.70	121.89	95.61	26.28	76.31

CHICKEN T-5.

	Gr. Corn meal Intake	Gr. Rice Intake	Total grams Intake	Total grams Outgo	Total grams Assimilated	Gr. Corn meal Digested	Gr. Rice Digested	Coefficient Rice
Organic matter	136.87	45.04	181.91	37.06	144.85	113.90	30.95	68.71
Crude protein	14.33	4.25	18.58	5.03	13.55	10.53	3.02	71.01
Ether extract	8.82	1.05	9.87	1.59	8.28	7.45	0.83	79.26
Crude fiber	4.57	5.30	9.87	9.25	0.62	0.31	0.31	5.87
Nitrogen free extract	109.15	34.44	143.59	21.19	122.40	95.61	26.79	77.80

TOTALS AND AVERAGES OF CHICKENS T-1, -2, -3, -4, AND -5.

RICE ONLY

	Total Intake grams	Total Outgo grams	Total Assimilation grams	Coefficient
Organic matter	225.20	68.00	157.20	69.17
Crude protein	21.25	5.72	15.53	73.08
Ether extract	5.25	1.05	4.20	80.00
Crude fiber	26.50	24.96	1.54	5.81
Nitrogen free extract	172.20	36.27	135.93	78.93

CHICKEN U-1. RATION: CORN MEAL ¾, KAFIR CORN ¼.

	Gr. Corn Meal Intake	Gr. Kafir Corn Intake	Total grams Intake	Total grams Outgo	Total grams Assimilated	Gr. Corn Meal Digested	Gr. Kafir Corn Digested	Coefficient Kafir Corn
Organic matter	156.42	52.14	208.56	35.56	173.00	130.16	42.84	82.16
Crude protein	16.38	6.54	22.92	6.42	16.50	12.03	4.47	68.40
Ether extract	10.08	1.92	12.00	1.89	10.11	8.51	1.60	83.69
Crude fiber	5.22	1.80	7.02	6.59	0.43	0.35	0.08	4.19
Nitrogen free extract	124.74	41.88	166.62	20.66	145.96	109.27	36.69	87.61

CHICKEN U-2.

Organic matter	156.42	52.14	208.56	37.23	171.33	130.16	41.17	78.95
Crude protein	16.38	6.54	22.92	6.30	16.62	12.03	4.59	70.18
Ether extract	10.08	1.92	12.00	1.09	9.91	8.51	1.40	72.86
Crude fiber	5.22	1.80	7.02	6.58	0.44	0.35	0.09	5.13
Nitrogen free extract	124.74	41.88	166.62	22.26	144.36	109.27	35.09	83.79

CHICKEN U-3.

Organic matter	156.42	52.14	208.56	38.40	170.16	130.16	40.00	76.71
Crude protein	16.38	6.54	22.92	6.53	16.39	12.03	4.36	67.21
Ether extract	10.08	1.92	12.00	2.11	9.89	8.51	1.38	71.86
Crude fiber	5.22	1.80	7.02	6.59	0.43	0.35	0.08	4.81
Nitrogen free extract	124.74	41.88	166.62	23.17	143.45	109.27	34.18	81.62

Chicken U-4. Ration: Corn Meal ¾, Kafir Corn ¼.

	Gr. Corn Meal Intake	Gr. Kafir Corn Intake	Total grams Intake	Total grams Outgo	Total grams Assimilated	Gr. Corn Meal Digested	Gr. Kafir Corn Digested	Coefficient Kafir Corn
Organic matter	156.42	52.14	208.56	38.97	169.59	130.16	39.43	75.62
Crude protein	16.38	6.54	22.92	6.43	16.49	12.03	4.46	68.26
Ether extract	10.03	1.92	12.00	2.14	9.86	8.51	1.35	70.19
Crude fiber	5.22	1.80	7.02	6.60	0.42	0.35	0.07	3.98
Nitrogen free extract	124.74	41.88	166.62	23.80	142.82	109.27	33.55	80.11

Chicken U-5.

	Gr. Corn Meal Intake	Gr. Kafir Corn Intake	Total grams Intake	Total grams Outgo	Total grams Assimilated	Gr. Corn Meal Digested	Gr. Kafir Corn Digested	Coefficient Kafir Corn
Organic matter	156.42	52.14	208.56	36.60	171.96	130.16	41.80	80.11
Crude protein	16.38	6.54	22.92	6.35	16.57	12.03	4.54	69.49
Ether extract	10.08	1.92	12.00	1.96	10.04	8.51	1.53	79.86
Crude fiber	5.22	1.80	7.02	6.59	0.43	0.35	.03	4.17

Totals and Averages of Chickens U-1, -2, -3, -4, and -5.

Kafir Corn Only

	Total Intake grams	Total Outgo grams	Total Assimilated grams	Coefficient
Organic matter	260.70	55.46	205.24	78.72
Crude protein	32.70	10.28	22.42	68.56
Ether extract	9.60	2.34	7.26	75.61
Crude fiber	9.00	8.60	0.40	4.45
Nitrogen free extract	209.40	34.24	175.16	83.65

CHICKEN V-1. RATION: CORN MEAL ¾, BARLEY ¼.

	Gr. Corn Meal Intake	Grams Barley Intake	Total grams Intake	Total grams Outgo	Total grams Assimilation	Gr. Corn Meal Digested	Grams Barley Digested	Coefficient Barley
Organic matter	156.42	52.02	208.44	38.20	170.24	130.16	40.08	75.36
Crude protein	16.38	6.54	22.92	5.96	16.96	12.03	4.93	75.36
Ether extract	10.08	1.74	11.82	2.15	9.67	8.51	1.16	66.83
Crude fiber	5.22	3.00	8.22	7.73	0.49	0.35	0.14	4.71
Nitrogen free extract	124.74	40.74	165.48	22.36	143.12	109.27	33.85	83.10

CHICKEN V-2.

	Gr. Corn Meal Intake	Grams Barley Intake	Total grams Intake	Total grams Outgo	Total grams Assimilation	Gr. Corn Meal Digested	Grams Barley Digested	Coefficient Barley
Organic matter	156.42	52.02	208.44	39.96	168.48	130.16	38.32	73.66
Crude protein	16.38	6.54	22.92	6.30	16.62	12.03	4.59	70.14
Ether extract	10.08	1.74	11.82	2.23	9.59	8.51	1.08	61.93
Crude fiber	5.22	3.00	8.22	7.75	0.47	0.35	0.12	3.98
Nitrogen free extract	124.74	40.74	165.48	23.68	141.80	109.27	32.53	79.87

TOTALS AND AVERAGES FOR CHICKENS V-1, AND -2.

BARLEY ONLY

	Total Intake grams	Total Outgo grams	Total Assimilated grams	Coefficient
Organic matter	104.04	25.64	78.40	75.35
Crude protein	13.08	3.56	9.52	72.77
Ether extract	3.48	1.24	2.24	64.36
Crude fiber	6.00	5.74	0.26	4.33
Nitrogen free extract	81.48	15.10	66.38	81.46

CHICKEN W-1. RATION: CORN MEAL ¾, WHEAT MIDDLINGS ¼.

	Gr. Corn Meal Intake	Gr. Wheat Middlings Intake	Total grams Intake	Total grams Outgo	Total grams Assimilation	Gr. Corn Meal Digested	Gr. Wheat Middlings Digested	Coefficient Wheat Middlings
Organic matter	156.42	51.84	208.26	48.33	159.93	130.16	29.77	57.42
Crude protein	16.38	10.14	26.52	6.87	19.65	12.03	7.62	75.18
Ether extract	10.08	3.06	13.14	2.52	10.62	8.51	2.13	69.61
Crude fiber	5.22	5.10	10.32	9.78	0.54	0.35	0.19	3.69
Nitrogen free extract	124.74	33.54	158.28	29.18	129.10	109.27	19.83	59.12

CHICKEN W-2.

Organic matter	156.42	51.84	208.26	46.39	161.87	130.16	31.71	61.16
Crude protein	16.38	10.14	26.52	6.40	20.12	12.03	8.09	79.82
Ether extract	10.08	3.06	13.14	2.40	10.74	8.51	2.23	73.18
Crude fiber	5.22	5.10	10.32	9.75	0.57	0.35	0.22	4.27
Nitrogen free extract	124.74	33.54	158.28	27.84	130.44	109.27	21.17	63.13

CHICKEN W-3.

Organic matter	156.42	51.84	208.26	46.64	161.62	130.16	31.46	60.66
Crude protein	16.38	10.14	26.52	6.60	19.92	12.03	7.89	77.81
Ether extract	10.08	3.06	13.14	2.36	10.78	8.51	2.27	74.26
Crude fiber	5.22	5.10	10.32	9.76	0.56	0.35	0.21	4.42
Nitrogen free extract	124.74	33.54	158.28	27.92	130.36	109.27	21.09	62.89

TOTALS AND AVERAGES FOR CHICKENS W-1, -2, AND -3.

WHEAT MIDDLINGS ONLY

	Total Intake grams	Total Outgo grams	Total Assimilated grams	Coefficient
Organic matter	155.52	62.58	92.94	59.78
Crude protein	30.42	6.82	23.60	77.57
Ether extract	9.18	2.55	6.63	72.22
Crude fiber	15.30	14.68	0.62	4.05
Nitrogen free extract	100.62	38.53	62.09	61.70

MONOGRAPHIC TABLES OF DIGESTIVE COEFFICIENTS WITH POULTRY

COMPOSITION OF POULTRY FEEDS—ALL ANALYSES TO DATE

TABLE VII

Feed	Water	Ash	Crude Protein	Fiber	N.F.Ex	Fat	No. Analysis
Barley	9.31	2.70	11.49	4.59	69.82	2.11	302
Buckwheat	11.90	2.21	10.84	10.10	62.24	2.71	21
Clover, red (Green)	73.80	2.10	4.10	7.30	11.70	1.00	85
Corn	12.01	1.51	10.39	1.80	69.28	5.01	58
Corn, cracked*	10.29	1.61	10.30	2.00	70.79	5.01	5
Corn meal, bolted*	13.13	1.23	10.10	1.79	68.10	5.65	6
Corn meal, unbolted*	11.10	2.00	9.10	2.90	69.30	5.60	4
Corn meal	11.30	1.30	9.30	2.30	72.00	3.80	7
Cottonseed meal	7.91	6.40	37.60	11.50	28.37	8.20	486
Cowpeas	11.60	3.40	23.60	4.10	55.80	1.50	11
Oats	9.21	3.41	12.39	10.10	61.50	3.39	498
Oats, hulled*	8.20	3.10	13.90	1.90	64.20	8.70	5
Oats, rolled	7.90	2.00	16.00	1.50	66.10	6.50	8
Kafir corn	11.35	1.78	11.02	2.33	70.52	3.00	141
Millet	9.10	3.30	11.80	7.80	64.70	3.30	6
Potatoes, raw	78.80	1.10	2.20	0.40	17.40	0.10	465
Peanut meal (Fat not extracted)*	6.10	4.90	21.30	16.60	15.50	35.60	4
Peas	9.20	3.40	22.90	5.60	57.80	1.10	6
Rice	9.33	5.05	7.78	9.60	66.34	1.90	16
Rye	9.43	2.00	11.80	1.86	73.10	1.81	112
Soybean meal	11.12	5.38	41.66	5.46	28.90	7.48	10
Wheat	10.20	1.90	12.41	2.20	71.19	2.10	864
Wheat bran	10.10	6.30	16.00	9.50	53.70	4.40	7,742
Wheat middlings*	9.29	4.34	15.72	7.15	59.00	4.50	10
Meat scrap	7.50	16.60	59.30	---------	5.00	11.60	31
Blood meal	9.64	3.34	82.38	---------	3.77	0.87	50
Buttermilk (dried)*	10.12	6.42	34.16	---------	48.31	0.99	4
Fish meal*	9.70	31.10	49.30	---------	---------	9.90	4
Meat and bone meal	5.90	36.36	39.69	2.09	4.89	11.37	63
Digester tankage	7.42	19.29	52.15	3.11	4.03	14.00	57

*Analyses by Kaupp and Ivey, only.

KAUPP-IVEY TABLE

Showing Average Digestive Coefficients, for Poultry, to Date

TABLE VIII

Feed	Number of Trials	Organic Matter	Crude Protein	Fat	Fiber	Nitrogen Free Extract
Wheat middlings	18	52.55	64.52	53.59	8.45	54.81
Corn	18	86.43	81.05	83.23	5.86	90.55
Wheat	16	80.68	69.82	48.78	6.40	87.73
Oats	19	63.52	73.83	80.22	11.69	74.37
Corn meal (bolted)	5	84.67	74.04	86.26	6.66	88.18
Meat and bone meal	2	86.82	92.17	93.13	3.23	76.56
Tankage	2	85.50	90.70	91.71	3.94	81.88
Blood meal	4	86.71	88.10	78.36	----------	71.55
Buttermilk (dried)	5	80.79	81.55	78.01	----------	81.14
Soybean meal (Fat extracted)	5	77.61	83.33	81.41	2.16	83.14
Peanut meal (Fat not extracted)	5	65.94	80.33	78.41	4.08	84.14
Cottonseed meal	4	73.32	81.94	79.05	5.46	82.92
Corn meal (unbolted)	3	83.21	73.50	84.50	6.80	87.60
Rye	4	77.65	71.56	25.05	4.68	84.19
Hulled oats	5	81.75	69.83	84.57	4.91	86.22
Buckwheat	8	70.14	56.79	85.09	6.33	82.69
Rice	5	69.17	73.08	80.00	5.81	78.93
Kafir corn	11	83.50	68.56	76.96	4.45	90.60
Barley	5	76.46	75.90	66.45	4.33	84.52
Corn, cracked	2	83.30	72.20	87.10	----------	88.10
Wheat bran	3	46.70	71.70	37.00	----------	46.00
India wheat	3	72.70	75.00	83.80	----------	83.40
Oats, rolled	4	89.30	80.10	92.20	----------	94.30
Peas	3	76.60	88.10	81.20	----------	87.10
Cowpeas	6	71.90	----------	88.70	----------	87.50
Millet	2	----------	62.40	85.70	----------	98.40
Potatoes	6	78.30	46.90	----------	----------	84.50
Beef scrap	4	83.60	91.40	91.00	----------	----------
Fish meal	5	91.60	91.48	92.24	----------	----------
Corn meal	5	84.20	74.60	89.90	----------	89.30
Clover, (calculated)	3	27.70	70.60	35.50	----------	14.30

KAUPP-IVEY TABLE OF AVERAGE DIGESTIBLE NUTRIENTS OF POULTRY FEEDS

Based on digestive experiments with poultry as determined by B. F. Kaupp and J. E. Ivey, N. C. Experiment Station, Brown, U. S. Department of Agriculture, Bartlett, Maine Experiment Station, Fields and Ford, Oklahoma Experiment Station, and Paraschtsehuk, Kaluginand, and Grost.

TABLE IX

DIGESTIBLE NUTRIENTS IN 100 POUNDS

Feed	Total Dry Matter	Crude Protein	Fiber	Nitrogen Free Extract	Fat	Total Pounds
Barley	90.69	8.70	0.18	58.94	1.40	69.22
Buckwheat	88.10	6.14	0.64	51.47	2.30	60.55
Clover, red (green)	16.20	2.89	----------	1.67	0.35	4.91
Corn	87.81	8.42	0.10	62.73	4.17	75.42
Corn, cracked	89.71	7.44	----------	62.54	4.36	74.34
Corn meal, bolted	86.87	7.47	0.12	60.05	4.87	72.51
Corn meal, unbolted	88.90	6.69	0.19	60.53	4.73	72.14
Corn meal	88.70	6.93	----------	64.30	3.41	74.64
Cottonseed meal	92.09	27.63	0.78	23.54	6.93	58.88
Cowpeas	88.40	----------	----------	48.82	1.33	50.15
Oats	80.79	9.77	1.28	45.73	2.72	59.50
Oats, hulled	91.80	9.70	0.09	55.35	7.36	72.50
Oats, rolled	92.10	12.82	----------	62.33	5.99	81.14
Kafir corn	88.65	7.56	0.10	63.89	2.31	73.86
Millet	90.90	7.36	----------	63.66	2.83	73.85
Potatoes	21.20	1.03	----------	14.71	----------	15.74
Peanut meal (Fat not extracted)	93.90	17.11	0.68	13.04	27.91	58.74
Peas	90.80	20.17	----------	50.34	0.89	71.40
Rice	90.67	5.69	0.56	52.36	1.52	60.13
Rye	90.57	8.44	0.09	61.54	0.46	70.53
Soybean meal (Fat extracted)	88.88	34.71	0.11	24.02	6.09	64.93
Wheat	89.80	8.66	0.14	62.45	1.02	72.27
Wheat bran	89.90	11.47	----------	24.70	1.63	37.80
Wheat middlings	90.71	10.14	0.60	32.33	2.41	45.48
Meat meal	92.50	54.20	----------	----------	10.56	64.76
Blood meal	90.26	72.58	----------	2.70	0.68	75.96
Buttermilk (dried)	89.88	27.86	----------	39.20	0.77	67.83
Fish meal	90.30	45.10	----------	----------	9.13	54.23
Meat and bone meal	94.10	36.58	0.07	3.74	10.59	50.98
Digester tankage	92.58	47.30	0.09	3.08	12.84	63.31

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JUNE 1923 TECHNICAL BULLETIN No. 23

Hybridization of Vitis Rotundifolia

Inheritance of Anatomical Stem Characters

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NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
Conducted Jointly by the
STATE DEPARTMENT OF AGRICULTURE
and the
NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING
RALEIGH

BULLETINS OF THE STATION WILL BE SENT FREE TO CITIZENS OF THE STATE

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ILLUSTRATIONS

(Grouped at back of this Bulletin)

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Fig. 2. Vitis vinifera, longitudinal section through the node.

Fig. 3. Vitis rotundifolia, transverse section of internode.

Fig. 4. Vitis rotundifolia, transverse section of bundle, (*p*) phellogen, (*f*) sclerenchyma fibers, (*h*) hard bast, (*s*) soft bast, (*c*) cambium, (*r*) ray, (*v*) vessel.

Fig. 5. Vitis vinifera, transverse section of internode.

Fig. 6. Vitis vinifera, transverse section of bundle, (*p*) phellogen, (*f*) sclerenchyma fibers, (*h*) hard bast, (*s*) soft bast, (*c*) cambium, (*r*) ray, (*v*) vessel.

Fig. 7. Hybrid (V. vinifero x V. rotundifolia), transverse section of bundle.

Fig. 8. Hybrid (V. vinifera x V. rotundifolia)', transverse section of bundle.

Fig. 9. Hybrid (V. vinifera x V. rotundifolia), transverse section of bundle.

Fig. 10. Hybrid (V. vinifera x V. rotundifolia), transverse section of bundle.

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Fig. 45. V. bourquiniana, transverse section of bundles.

Fig. 46. Variety Winchell, transverse section of internode.

Fig. 47. Variety Winchell, transverse section of bundles.

Fig. 48-75. Camera lucida diagrams of hybrids of Winchell x V. rotundifolia.

Fig. 76. Hybrid (var. Winchell x V. rotundifolia), transverse section of bundles.

Fig. 77. Hybrid (var. Winchell x V. rotundifolia), transverse section of bundles.

Fig. 78. Hybrid (var. Winchell x V. rotundifolia), transverse section of internode.

HYBRIDIZATION OF VITIS ROTUNDIFOLIA

INHERITANCE OF ANATOMICAL STEM CHARACTERS

By C. F. Williams, *Assistant*
Division of Horticulture

In plant breeding, especially of horticultural crops, little or no attention has been paid to anatomical characters. Only those of weight, size, color, etc., and such physiological characters as sugar, starch, and protein content have been studied. In fact, rarely is a cross effected in which the anatomy of the parents differs sufficiently to make possible any comparison. However, in hybridizing *Vitis rotundifolia* with other species of Vitis, individuals with considerable difference in anatomy have been successfully crossed and the hybrid vines obtained are of interest from this viewpoint. It is possible to examine the hybrid stems not only for such superficial characters as smoothness, color, hardness, size, etc., but also for the fundamental anatomical structure which is the basis of these, and therefore the critical point of attack for a genetical study.

Vitis rotundifolia, the southern muscadine grape, differs considerably from other species of the genus Vitis, especially in certain of the stem characters. Gray (1) has divided the genus Vitis into two sub-genera. Under one, Muscadinia (Planch.) he places *V. rotundifolia* and under the other, Euvitis (Planch.), he includes all other grapes. Small (2) has reserved the genus Vitis for the bunch grapes proper and makes a separate genus for the muscadine grape, namely Muscadinia (Small). This will give some idea of the extent to which *V. rotundifolia* species differ from the other species of Vitis. Only those differences that affect the material under discussion will be considered here.

The hybrid vines on which the following report is based are some grown in determining the limits of hybridization of *Vitis rotundifolia* with other species of Vitis. The particular crosses used are as follows:

TABLE 1. PARENTAGE OF HYBRIDS

Year Made	No. of Vines	Description of Cross
1917	26	V. vinifera (var. Malaga) x V. rotundifolia
1916	7	V. bourquiniana (var. Herbemont) x V. rotundifolia
1912-16-18	26	Hybrid (Labrusca x Aestivalis x Vinifera var. Winchell) x V. rotundifolia.
1917	1	Hybrid (Winchell x V. rotundifolia) x V. rotundifolia

This does not mean that these are the only crosses of *V. rotundifolia* that have been secured. Other hybrids obtained have not been used because of insufficient growth in some cases, and death of the vines in others.

These vines have already been described as to their external characters by Detjen (3). This paper is limited to the gross anatomy of the mature one year canes of the parent vines and their F_1 generation hybrids. Material was collected during the early winter of 1921-22, and again in the winter of 1922-23. In order to have a uniform basis of comparison, only mature one year canes were used. Transverse, radial, and tangential sections were cut 25-30 microns thick, from the middle of the internode with a table microtome. Combination stains of safranin with Delafield's hematoxylin or with light green were used. Table 2 lists the different species and varieties of Vitis examined during this investigation.

TABLE 2. SPECIES OF VITIS EXAMINED

SPECIES	VARIETY	NO. OF VINES
Rotundifolia	Pure species and varieties	12
Vinifera	Malaga	3
Bourquinana	Herbemont	2
Labrusca	Concord	2
Labrusca	Pure species	3
Cinerea	Pure species	1
Arizonica	Pure species	1
Californica	Pure species	1
Aestivalis	Pure species	5
Aestivalis	Norton	1
Munsoniana	Pure species	1
Champini	Pure species	1
Bicolor	Pure species	1
Simpsoni	Pure species	1
Doaniana	Pure species	1
Hybrid	(Labrusca x Aestivalis x Vinifera) Winchell	2
Hybrid	(var. Winchell x V. rotundifolia)	26
Hybrid	(V. vinifera x V. rotundifolia)	26
Hybrid	(V. bourquiniana x V. rotundifolia)	4
Hybrid	[Hybrid (Winchell x V. rotundifolia) x V. rotundifolia.]	1

VITIS ROTUNDIFOLIA

Bark. The bark of *V. rotundifolia* is greenish-grey in color, rather smooth, and has numerous lenticels. It is very persistent, never shedding on the young wood, and on the very old wood falling off in small corky flakes. The stems of the young vines resemble those of maple saplings much more than they do the vines of the Euvitis group.

Wood. The wood is very hard and compact, the specific gravity being about 1.26 with the result that the stems will sink in water. The hardness of the wood is especially noticeable in pruning as the stems are much more difficult to cut than any Euvitis species.

Pith. The diameter of the pith column is very small, particularly as compared with other species of Vitis, but widens out at the node

(Fig. 1), at which there is no diaphragm. The pith is light green in color and so dense that Munson (4) in describing it says, "in place of pith a dense, dark green, cellular, nonfibrous wood." The cells are uniform in size in the node and internode, very thick walled and pitted. Starch is stored throughout the pith column.

Xylem. In *V. rotundifolia* the xylem forms a greater part of the stem than any other species (Fig. 3). Moreover, it is distinguished from Euvitis species by the character of the wood, which is composed of numerous tracheæ or vessels embedded in a mass of wood fibers (Fig. 4), both of which are smaller in diameter than those of other species, except *V. munsoniana.* These fibers, which are almost round, are septate and have extremely thick walls and a very small lumen. They are lignified to a greater degree than Euvitis species as is seen in the difficulty in cutting the stems, in the staining reaction, and in the thick walls.

The vessels are of considerable diameter (Fig. 4), although the smallest to be found in Vitis species, and very long, due to many of the cross walls having been dissolved away. The pitting consists of elongated horizontal slits closed by thin membranes, as is common among vines. These pits are of two sizes depending on whether the vessel is in contact with wood fibers or with other vessels. Where the tracheæ adjoin wood fibers, the pits are fairly small horizontal slits arranged in vertical rows. If one vessel is in contact with another, the slits are very long, extending almost the width of the vessel. Tyloses occur in the vessels, the occluding cells being thin walled, parenchymatous, and not pitted.

Phloem. The arrangement of the elements of the phloem (hard and soft bast) in the Muscadinia species differs from that in the Euvitis species. Figure 4 is a photomicrograph of a cross-section of a bundle showing the phloem and part of the xylem and wood rays. Here in *V. rotundifolia* the phloem as seen in transverse section is triangular in outline with the hard bast more or less radially disposed on the sides of the soft bast. That is, the portion of the cambium differentiating hard bast remains about the same, while the increased circumference due to growth is adjusted by an increase in the amount of cambium forming soft bast. This widening of the phloem accompanying the growth of the stem results in a triangular-shaped mass of this tissue. The hard bast is composed of thick walled septate wood fibers. The cells of the soft bast, which are small and thin walled, are somewhat irregularly disposed.

External to the phloem there is a small round bundle of sclerenchyma fibers forming the apex of the triangle mentioned above. These fibers are extremely long, nearly round, very thick walled with small lumen, and septate.

Rays. The rays of all Vitis species are of the compound wood ray type common in vines. These are several cells wide and extend longitudinally through the internode and are full of starch (Fig. 4). Those of *V. rotundifolia* are not as broad as the ones of the Euvitis group. The most significant characteristic as contrasting with the other Vitis species is that portion of the ray extending between the phloem bundles. In *V. rotundifolia* the ray in this region expands by cell multiplication to fill the widening gap between the phloem as the stem increases in circumference (Fig. 4). This is clearly evident in the arrangement of the cells which here are formed in tangential rows, contrasting with the radial rows of the ray proper.

Cortex. The cortex in *V. rotundifolia* is in sharp contrast with that of the bunch grape type (Fig. 4), and the seat of this difference lies in the phellogen or cork cambium. In the Muscadinia species the phellogen is formed immediately under the epidermis, and produces internally the phelloderm of varying thickness. This is composed of parenchymatous cells in irregular longitudinal rows. *V. rotundifolia* is further characterized by the presence of numerous lenticels which penetrate the cortex for a considerable depth opposite the ends of the rays (Fig. 4).

VITIS VINIFERA

Bark. The bark of *V. vinifera* is red-brown to greyish-brown in color on the young wood. It is finely striated and sheds in long fibrous plates.

Wood. The wood is very soft and porous. It is light in weight (specific gravity less than 1.0), quite brittle, and more or less oblong in cross section.

Pith. The diameter of the pith column in comparison with that of the stem is much greater than in the case of *V. rotundifolia.* Figure 5 of *V. vinifera,* which is of the same magnification as Figure 3 of *V. rotundifolia,* shows the proportion of the tissues in section. The pith cells of *V. vinifera,* as may be seen in a comparison of these two photomicrographs, are much larger and thinner walled. The pith is dry and light brown in color and contains little or no starch. At the node, there is a distinct diaphragm (Fig. 2) composed of thick walled lignified cells with pitted walls.

Xylem. The elements of the xylem (vessels and wood fibers) are larger in diameter than are those of *V. rotundifolia.* This is best seen by contrasting Figure 5 with Figure 3. The wood fibers are more angular in section and not as thick walled, nor as lignified, and are in more distinct radial rows (Fig. 6). There are more vessels and they are larger in this species than in *V. rotundifolia,* but the sculpturing is similar. Tyloses in the vessels are common in this species. One occluded vessel may be seen in cross section at the lower right in Figure 6 and several in Figure 5.

Phloem. The arrangement of the phloem in *V. vinifera* is typical for all Euvitis species. Figure 6 is of the phloem portion of a bundle of *V. vinifera* and should be contrasted with Figure 4 of *V. rotundifolia* which is of the same magnification. In transverse section it is almost square in outline and is composed of alternating tangential layers of hard and soft bast. Thus in contrast with the Muscadinia type, the cambium forms first a layer of soft bast and then a layer of hard bast, each several cells deep, and repeating this differentiation until there may be as many as six or seven pairs of layers. The cells are arranged in radial rows and are of larger size than those of *V. rotundifolia.* The hard bast cells are more angular in outline and thinner walled than in that species, and the soft bast cells are much larger. External to the phloem of the bundle is a semicircular column of sclerenchyma fibers. They contrast with those of *V. rotundifolia* in being arranged in larger bundles which are semicircular, in being larger in diameter and very angular, and thinner walled.

Rays. The rays of *V. vinifera* (Fig. 6) are compound wood rays as in other Vitis species, though considerably wider than those of *V. rotundifolia.* In contrast with these latter, they extend between the phloem bundles without widening through cell multiplication. Thus, the growth of the stem causes the cells to become pulled tangentially and distorted, especially at the ends of the rays.

Cortex. As the cortex of Euvitis species is shed, the appearance in the cross section of the mature one year wood is quite different from that of the Muscadinia species (Fig. 6). The phellogen forms immediately external to the phloem and inside of the sclerenchyma bundles. This layer of cork cells cuts off the water supply of the tissues outside of it, causing them to dry out and fall off. As the stem increases in circumference, there is no multiplication of cells as in *V. rotundifolia* so that the cells are much distorted and torn. Thus the cells in this region are very irregular and compacted. There are no lenticels present in this type of stem, the open ends of the rays serving this purpose and often at the time that the bark is shed, simulating them in appearance.

V. VINIFERA X V. ROTUNDIFOLIA HYBRIDS

Bark. Most of the hybrids of this cross have a light greyish-brown bark which is obscurely striated and has few lenticels, and sheds in small flakes rather than in fibrous plates as in *V. vinifera.* The appearance of the stems is rather smoother than that of either parent due to the smaller number of lenticels and a more obscure striation. The number of lenticels varies inversely with the extent to which the bark is shed, some vines having a more or less persistent bark with many lenticels, and others having a loose bark and fewer lenticels and more striations.

Wood. The wood is intermediate between the two parents and is usually somewhat brittle. It is not as hard as *V. rotundifolia* but seldom as soft as *V. vinifera.* The specific gravity in all but two cases was greater than 1.0, and hence fresh-cut wood sinks in water. In cross section the stem is more or less elliptical rather than oblong as the *V. vinifera* parent, but not often as round as in *V. rotundifolia.*

Pith. The diameter of the pith column of the hybrids is a little less than the average of the two parents and it varies in color from light green to light brown. The size of the pith cells at the internode is about the same as in *V. rotundifolia,* but those at the node vary in size with the extent to which there is an expression of a diaphragm. In a small percentage of the hybrids (two of the twenty-six examined) there was a distinct diaphragm at the node visible to the naked eye. In the others there was a tendency for the cells at the node to be compacted in a vertical direction. In order to arrive at some conclusion as to the extent of this compaction the number of cells in a given area in an average region of the longitudinal section was counted. The results are given below:

TABLE 3

VINE	NUMBER OF CELLS AT		RATIO
	NODE	INTERNODE	
Vinifera	227	89	2.54 : 1
Rotundifolia	135	136	1.00 : 1
Hybrid with diaphragm	138	40	3.45 : 1
Hybrid without diaphragm	139	88	1.58 : 1

The above table would indicate that the cells of the internode resemble the *V. rotundifolia* parent, and that the cells at the node are more or less influenced by the *V. vinifera* parent. It was also observed that the increase in the number of cells at the node was due to a lessening of their vertical height with little change in their horizontal diameter.

Xylem. The character of the xylem is intermediate between that of the parents. The hybrids vary, however, through all the range, one in fact going beyond *V. vinifera* in size of cells and number of vessels. The fibers are larger than in *V. rotundifolia* but not as angular as in *V. vinifera.* The tracheæ also are larger than those of *V. rotundifolia* and the sculpturing is that typical of Vitis species. Some of the variation in the size and number of vessels of the hybrids is shown in Figures 7-10, and 13-38.

Phloem. In hybrids the arrangement of the elements of the phloem as seen in the transverse section covers the ranges of both parents. There is, however, a greater resemblance to the staminate parent in the majority of cases. In most of the vines there is a radial row of hard bast of greater or less extent on the sides of the phloem bundle (Figs. 7 and 8). In addition to this, there is also a tendency for tangential layers of hard bast to appear in the bundle itself, either starting from one side or both sides and not reaching all the way across, or extending all the way across the bundle (Fig. 9). In only two instances did the bundle resemble that of Euvitis species, one of which is shown in Figure 10. It is remarkable that the pattern of the phloem bundle as seen in cross section varies in the same stem to a very considerable extent, showing a lack of fixation of this character in a given stem (see Figs. 13-38). Some of the vines, however, are quite uniform in their pattern. Inasmuch as the cambium determines the formation of hard or soft bast under unknown influences, in cases where the pattern in which these elements are laid down varies in the same stem, it would seem that the cambium of the stem must vary in its constitution.

The size of the cells of the hard and soft bast is intermediate, as is the thickness of their walls, and their shape. The sclerenchyma bundle is more *V. rotundifolia* in character, though usually of larger size than this parent.

Figures 11 to 38, inclusive, are camera lucida diagrams of the same magnification of *V. vinifera, V. rotundifolia,* and their hybrids. The hard bast of the phloem (also the bundle of sclerenchyma fibers) is shown stippled. Figures 13 to 38 show different degrees of intermediacy of character in the hybrids. Figures 13 and 15 show two adjacent bundles each with contrasting arrangements of the hard and soft bast. Such cases are quite common among the hybrids. Figures 14, 20, 25, and 33 show a progression in the range between the two types of arrangement. Only in Fgures 33 and 35 has the *V. vinifera* character approached dominance. Figure 34 shows the elements of the hard bast scattered indiscriminately through the soft bast.

Rays. The character of the intervascular ray varies with the character of the phloem. If the bundle is triangular as in *V. rotundifolia,* the ray immediately widens out by cell multiplication as in this parent (Fig. 7), but if the phloem bundle is broad and square the ray extends out without increasing in width as in *V. vinifera* (Fig. 10). In some cases the ray extends part of the depth of the bundle before widening out, showing most exactly the intermediate character (Figs. 8 and 9).

Cortex. The cortex in the hybrids shows less fixation of a definite character than any other part of the stem, varying from a close resemblance to the staminate parent to a similarity to the pistillate parent. In most cases, the cortex is very much like that of the *V. rotundifolia* species. Only the two that have the V. vinifera-like phloem have a cortex resembling that parent. In many of the vines there is a great variation in the same stem, one side being quite different from the other, or having patches of V. vinifera-like cortex with large, loose, torn cells outside of a cortex otherwise resembling *V. rotundifolia* (Fig. 8 and 9). In the examples illustrated in Figures 7 and 8 the type of cortex shown is not typical for the entire section of the stem but only for a small number of bundles, more striking because of their irregular occurrence.

The indeterminate location of the phellogen seems to be the cause of this variation. It appears in the photomicrographs as a whitish layer due to the stain washing out of the suberized tissues. Its position varies not only in different vines but usually in the same vine (Fig. 7). In some cases, it is just under the epidermis as in *V. rotundifolia.* In other cases, it is inside of the sclerenchyma bundle as in the other parent (Fig. 10). Quite often there is an isolated layer of suberized tissue surrounding only one bundle of sclerenchyma fibers (Fig. 29). Frequently all three of these positions for the phellogen are found in the same stem, and in some instances at the same point in the stem. Usually the cortex inside the cork cambium resembles the *V. rotundifolia* parent, and that outside the *V. vinifera* parent (Fig. 9). Lenticels were present only where the phellogen is immediately under the epidermis. This scattered distribution of the cork cambium is responsible for the exfoliation of the bark in small flakes.

In Figures 11 to 38 the phellogen is shown in solid black. Figures 11 and 12 represent the parents, and Figures 13 to 38 the hybrids, which show all degrees of the cork cambium character expressed in the parents. Bundles diagrammed are more or less typical for the particular stem, but do not by any means represent the condition of each bundle of the stem. In Figure 13, for example, the phellogen occurred outside of only a few bundles at this particular location which is used to illustrate the arrangement of hard and soft bast in the phloem. Such cases as shown in Figures 19, 24, 37, and similar ones, usually occurred only in small patches of the circumference of the transverse section. In Figure 31 isolated suberized cells are scattered through the cortex.

VITIS BOURQUINIANA, Variety Herbemont

The stem of *V. bourquiniana* so closely resembles that of *V. vinifera* that a detailed description of it is unnecessary in this place. If any difference exists in the characters in question it is perhaps that the cells have thinner walls (Fig. 45).

V. BOURQUINIANA X V. ROTUNDIFOLIA HYBRIDS

It was possible to examine only four of the hybrids of this cross. The expression of the intermediate character is similar to that in the *V. vinifera* hybrids. There is, however, no example of the phloem bundle resembling that of the Euvitis parent and the resemblance was predominantly toward the other parent. In view of the small number of vines studied, this was hardly significant.

Diagrams of these hybrids are shown in Figures 39 to 44, inclusive, Figures 39 and 40 being from one vine, and Figures 43 and 44 from another single vine. In these cases the bundles were not adjacent but came from different parts of the same transverse section. The pair in Figures 39 and 40 show well, not only the tendency to form alternate layers of hard and soft bast, but also (Fig. 40) the squaring up of the bundle due to the intervascular ray not widening out by cell multiplication. Figure 43 shows the varying amount of phellogen in the same stem. Figure 42 is of interest because of the small amount of hard bast fibers present.

VARIETY WINCHELL

(Hybrid of V. labrusca, V. æstivalis, V. vinifera)

While it has not been possible, as yet, to pick out distinctive specific characters of the stem on the basis on which this study is made for the different species of Vitis, the anatomical characters of Winchell resemble most closely of the three parents, those of *V. aestivalis,* and the differences noted in the following description vary from the *V. vinifera* species in that direction.

Bark. The bark is reddish-brown in color, striated and shedding in long fibrous plates.

Wood. The wood is soft and brittle and quite porous. It is light in weight and almost round in cross-section.

Pith. The pith column is very large in diameter and larger in proportion to the diameter of the stem than *V. vinifera* (Fig. 46). In this, it resembles the *V. labrusca* parent. The pith is dry and loose, light-brown in color, and composed of large, thin-walled cells. There is a thick diaphragm at the node composed of compact lignified pith cells with thick walls having simple pits.

Xylem. The xylem of this variety is quite like that of *V. vinifera,* but the bundle is not as deep in proportion to the stem diameter on account of the large pith. The cells are a little smaller and not as thick walled. (Fig. 46.)

Phloem. The phloem is in the typical arrangement for Euvitis species. The cells are more uniform in size and smaller than *V. vinifera.* The fibers of the sclerenchyma bundle differ very little from those of *V. vinifera* (Fig. 47).

Cortex. The cortex in this species differs but little from that of *V. vinifera* as above described.

V. VAR. WINCHELL X V. ROTUNDIFOLIA HYBRIDS

Bark. Most of the hybrids of this cross have a rather smooth, reddish-brown bark, darker in color than the *V. vinifera* hybrids. On these the bark sheds either in long fibrous plates or in smaller flakes. Others of the cross have a persistent bark which is lighter in color and more of a greyish-brown, with many lenticels.

Wood. The wood is soft and very brittle, snapping off easily at the node and only a little less easily at the internode. The section of the stem is almost round in outline.

Pith. The diameter of the pith varies widely between that of the two parents, which are extremes in either direction for size of all the vines examined. In color, some have a light brown pith and others light green as in *V. rotundifolia.* In this latter case, the pith is usually continuous without a distinct diaphragm at the node. The others have a more or less complete diaphragm. Of the twenty-six examined, eleven have definite diaphragms at the node visible without the use of the lens. In those with continuous pith the cells at the node were compacted and lignified to a varying extent.

Xylem. The xylem is more nearly like that of the variety Winchell, especially in the size and shape of cells, although the walls are thicker than those of this variety.

Phloem. In these hybrids there is a great variation in the pattern of the phloem as examined in the transverse section. In eleven of the twenty-six the hard and soft bast are disposed in a manner similar to that in the Euvitis species. In the others, there is more or less similarity to the staminate parent, although there is a greater tendency for the hard bast to form tangential layers across the bundle regardless of the shape of the bundle than in the case of *V. vinifera* hybrids. The bundle of sclerenchyma fibers varies with the type of phloem bundle. The square Winchell-like bundle is accompanied by a bundle of sclerenchyma fibers similar to the same species.

The camera lucida diagrams shown in Figures 48-75 inclusive, represent bundles from each of the hybrids of this cross; Figures 55 and 56 being from the same vine, and Figures 60 and 61 from another single vine. The range of the intermediate character of the hard bast is shown by Figures 49, 51, 53, 56, 60 and 66. This is also well shown in the one vine represented by Figures 55 and 56, or in Figure 62. The vines represented by Figures 55 and 58 are interesting because of the scarcity of hard bast present in the phloem.

Rays. The rays show the same correlation with the shape of the phloem tissue as in the case of the *V. vinifera* hybrids, that is, the intervascular ray expands by cell multiplication when the phloem bundle is sufficiently triangular in shape to cause a tangential strain (Fig. 76).

Cortex. The cortex of these hybrids is extremely irregular. The predominant character is the excessive amount of cork cambium at scattered points in the transverse section (Fig. 77). This lack of definite character for the phellogen is greater than in the case of the *V. vinifera* hybrids. In some instances, the entire thickness of the cortex seems to be completely of suberized tissue (Figs. 76 and 78).

The diagrams in Figures 48-75 illustrate the various amounts and arrangements of the cork cambium. The quantity varies from that shown in Figure 51 to that of Figure 58, and its position in relation to other tissues varies greatly in depth as shown in Figures 52 and 63. In Figures 68-74 inclusive, the drying out of the cells outside of the suberized layer was so complete that this portion of the cortex fell off either before or after cutting the section. In each of these cases, the phellogen was located inside of the sclerenchyma bundles.

HYBRIDS VAR. WINCHELL—V. ROTUNDIFOLIA HYBRID X V. ROTUNDIFOLIA

There is only one vine of this cross and it is so small and weak that it would be unwise to draw any conclusions from it. It is, however, predominantly *V. rotundifolia* in character, which, inasmuch as this is the staminate parent, would indicate its hybrid character. The stem and bark resemble this parent and the pith is small, light green and continuous. The transverse section of the stem shows strongly the *V. rotundifolia* parentage in the pattern of the phloem bundle, the proportion of elements and the presence of lenticels. At one point of this section there is an excessive amount of dry cortex outside of the phelloderm resembling the hybrids of the F_1 generation.

DISCUSSION

It has been possible in the hybridization of Vitis species to cross individuals that have considerable difference in the anatomy of the stem structure. These characters are inherited by the hybrid offspring so that there is an opportunity to study the inheritance of characters that are of greater fundamental importance from a genetical viewpoint than such superficial ones as color, weight, size, etc. No doubt, some of these characters may be correlated with other external morphological ones, but the study of the inheritance of these essential structural characters should be of great value to an understanding of the principles of heredity.

The varying degree of expression of the blended intermediate character in the offspring would probably be best explained by a multiple factor hypothesis. The small number of individuals available and the fact that only the F_1 generation has been studied would make it impossible to draw any definite conclusions.

It is very significant, however, that the intermediate condition of the anatomical stem characters varies in degree not only in the different hybrid vines, but also in the same individual. It is a striking fact that neighboring and even adjacent bundles in the same transverse section may resemble different parents. In such small stems, this condition cannot be explained as a response to external environmental factors. This condition approaches a chimera but differs from it in the lack of total dominance of expression of either parent. Actually there are different degrees of blending in the same individual. There are here several interdependent characters. Changes affecting one of them therefore will to some extent influence the others. Where the degree of inheritance of the intermediate condition is uniform for each character of the individual, there is a stem that is fairly uniform in its composition. On the other hand, where the degree of inheritance of the different characters varies for the individual, the influence of the related characters results in a complex condition. It is necessary to consider this on the basis of the generative tissue of the cambium. It would appear that the cambium varies in its hereditary composition, a difficult thing to explain or believe.

It would probably be advisable if possible to determine the immediate causes influencing the formation of hard bast in the phloem, and the formation of phellogen. Knowing these influences, it might be possible to find a hereditary factor or factors which would control these characters.

In regard to the formation of phellogen, Douliot (5) finds that the cork in the stem may be superficial, pericylic or intermediate in origin. In the parents of the crosses described here, the Euvitis species have the cork cambium pericylic, and the Muscadinia species superficial. However, the lack of an intermediate condition for this character in the hybrids of the F_1 generation but rather a proliferation of suberized tissue would indicate the inheritance of a tendency to form cork cambium in both locations.

Priestley (6) has studied the formation of phellogen from the casual standpoint, finding that it is related to "1, the blocking of a parenchymatous surface usually by a deposit of suberin or cutin formed in the presence of air; 2, the accumulation of sap at the parenchymatous surface thus blocked; 3, the consequent development and activity of a phellogen amidst this parenchyma." This would hold true in the case of the parents of these hybrids. In Muscadinia species the sap can move outward in the stem to the epidermis where it is blocked and phellogen subsequently formed. In the Euvitis stem the cortical cells are destroyed during the expansion of the stem and air enters as deep as the endodermis and the cork cambium is formed here. In the hybrids where a combination of these conditions exist, it is difficult to explain phellogen formation as due to these causes, especially in those cases where there is a layer of isolated phellogen around a bundle of sclerenchyma fibers, or where there are two layers of phellogen. In other cases it would appear that the location of the cork cambium depended on the ability of the cortical cells to multiply and thus present a layer of parenchymous cells impervious to the air. Such an explanation practically removes the phellogen from direct inheritance by hereditary factors, the hereditary factors controlling only the cambium and the tissues formed by it, and the phellogen formation being controlled by air and moisture.

The significance of the different degree of intermediate inheritance in the hybrids of *V. rotundifolia* with *V. vinifera* var. Malaga, and with Vitis hybrid var. Winchell has not been determined. The difference between *V. rotundifolia* and each of these is apparently about the same as far as actual characters are concerned. Whether the genetical difference is greater in case of var. Winchell crosses due possibly to its own hybrid nature, is still to be investigated. Unfortunately, individuals of crosses between *V. rotundifolia* and each of the species represented in the pedigree of Winchell cannot at this time be examined. A study of *V. labrusca* and *V. æstivalis* has given no additional information.

This study of the anatomy of the stems of Vitis species probably explains the difficulty of rooting Muscadine cuttings, and grafting *V. vinifera* on Muscadinia species roots. It may be possible to develop a hybrid root resistant to phylloxera and on which the *V. vinifera* may be easily grafted.

SUMMARY

The stems of the subgenera of Vitis, namely, Muscadinia and Euvitis, have distinctly different anatomical characters.

These characters may be summarized as follows:

Character	*Muscadinia*	*Euvitis*
Bark	persistent	shedding
Lenticels	present	absent
Pith	small, of thick-walled cells	large, of thin-walled cells
Diaphragm	absent at node	present at node
Wood fillers	small diameter, thick-walled	larger, thin-walled
Phloem section	triangular	square
Hard bast	outlines soft radially	in alternate layers with soft fibers
Sclerenchyma fibers	small bundle of round thick-walled fibers	large bundle of angular fibers
Rays in intervascular region	widen by cell increase	continue the same width
Phelloderm	present	absent
Phellogen	immediately under epidermis	just outside of phloem
Sp. gravity of fresh cut wood	greater than water	less than water

In crosses between the species of these subgenera, the hybrids inherit the anatomical characters as well as the morphological characters, and just as strikingly as the external characters usually studied.

These characters in the hybrids were intermediate resembling either parent with varying degree, but with possibly greater resemblance to the *V. rotundifolia* parent.

In the hybrids with var. Winchell there was less resemblance to the *V. rotundifolia* parent than in the *V. vinifera* hybrids.

The degree of the expression of the intermediate condition was about uniform in all the characters of any one vine. That is, a vine with more resemblance to one parent in any character had more resemblance to that parent in the other characters.

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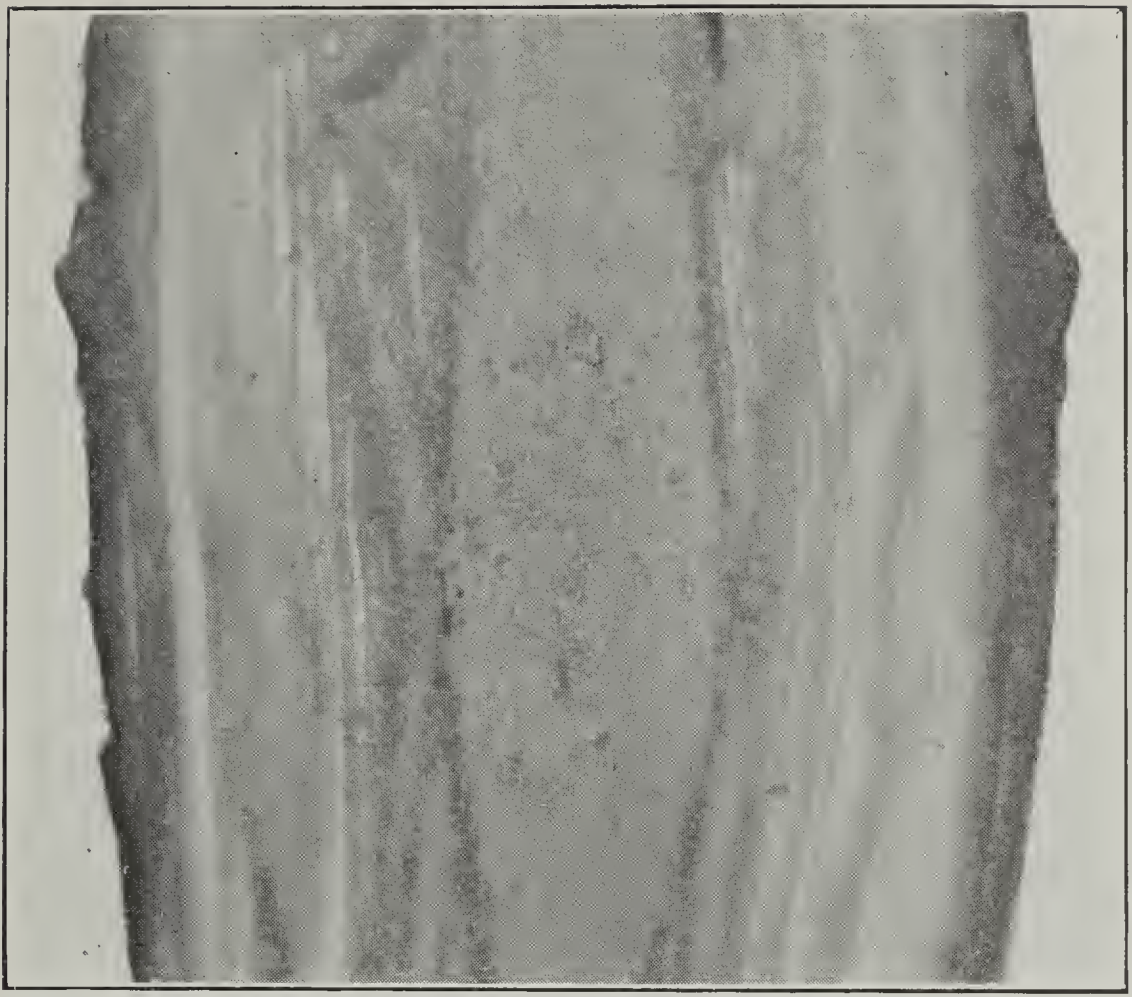

FIG. 1. VITIS ROTUNDIFOLIA, LONGITUDINAL SECTION THROUGH THE NODE

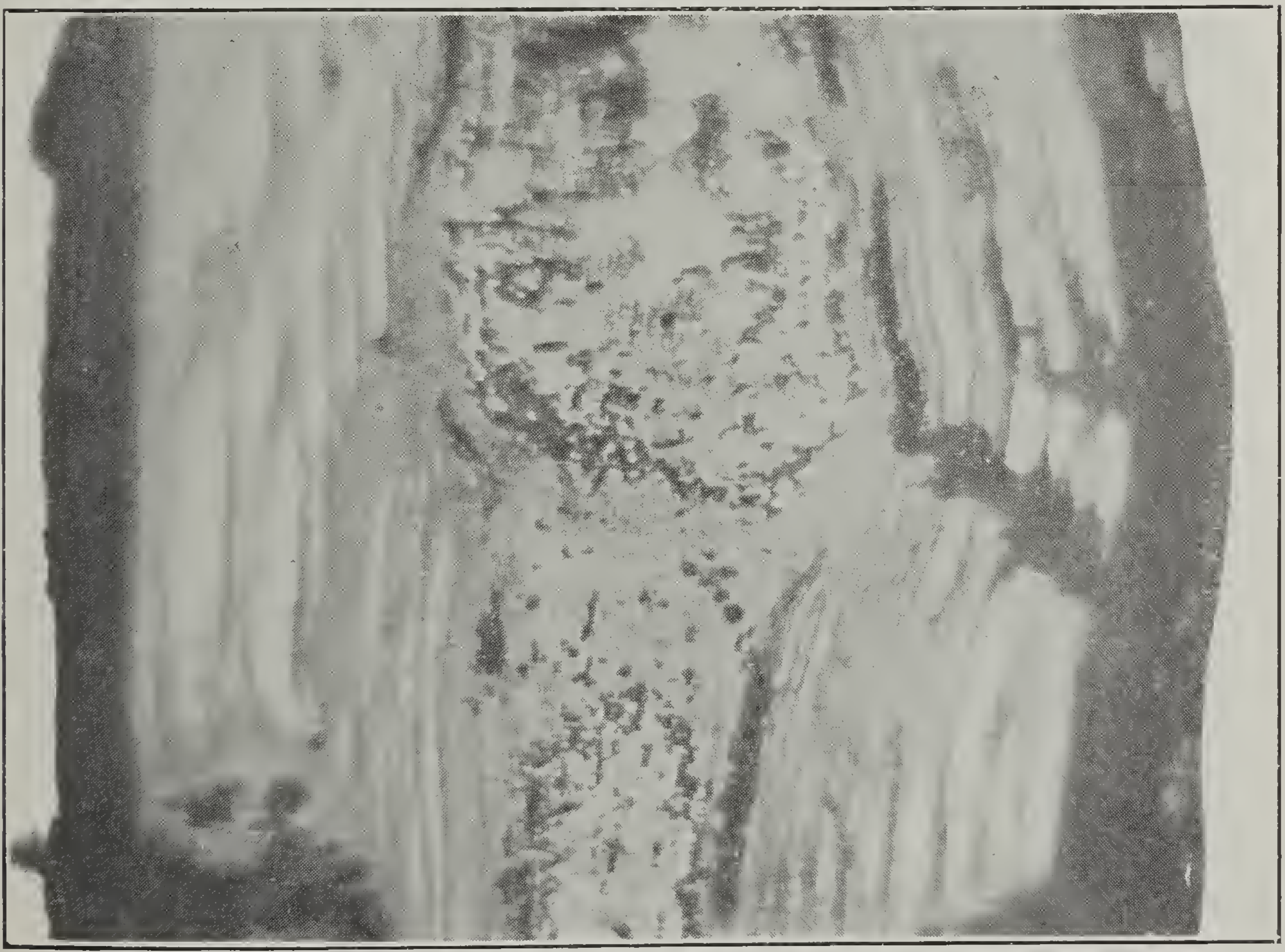

FIG. 2. VITIS VINIFERA, LONGITUDINAL SECTION THROUGH THE NODE.

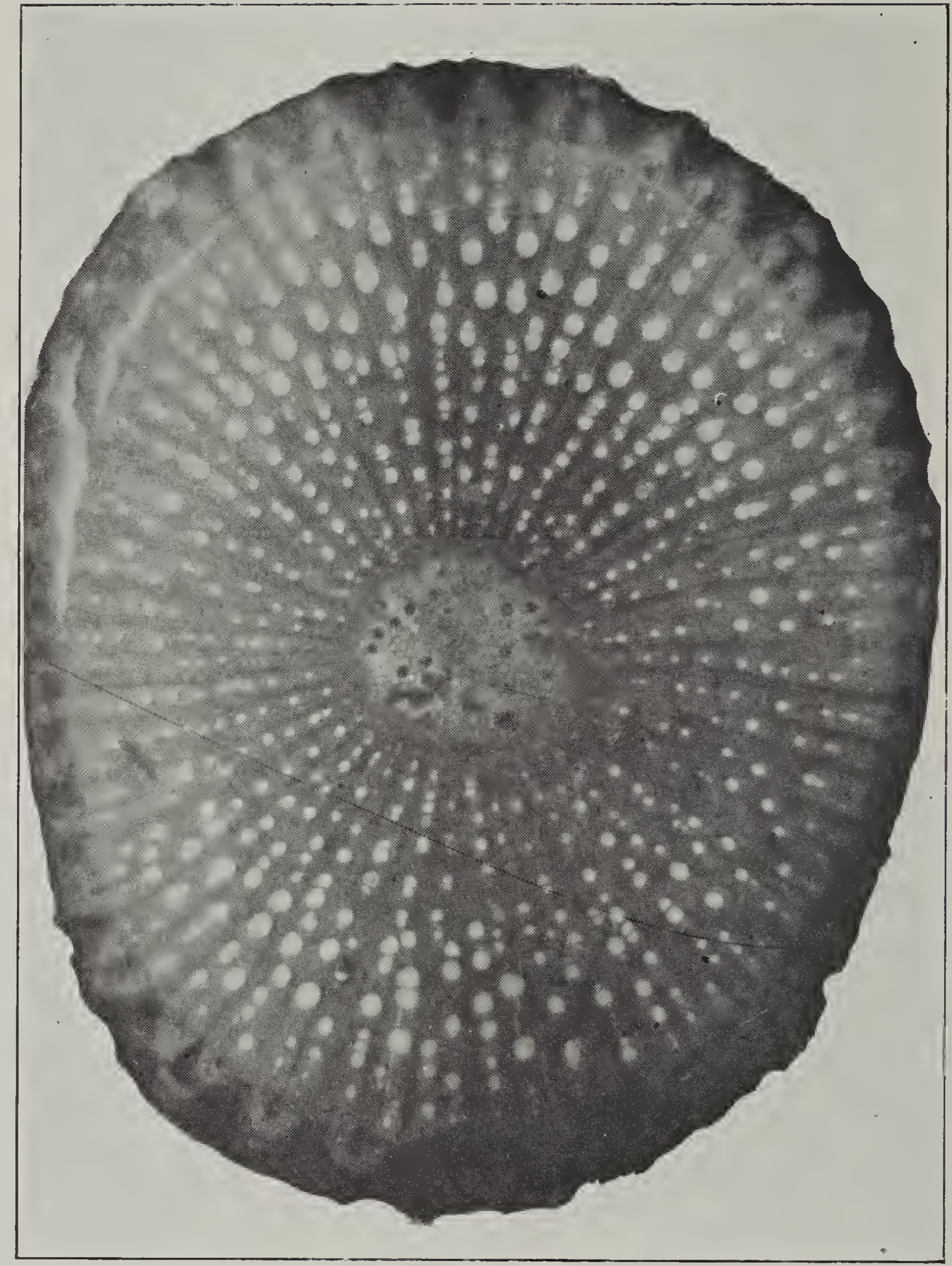

FIG. 3. VITIS ROTUNDIFOLIA, TRANSVERSE SECTION OF INTERNODE.

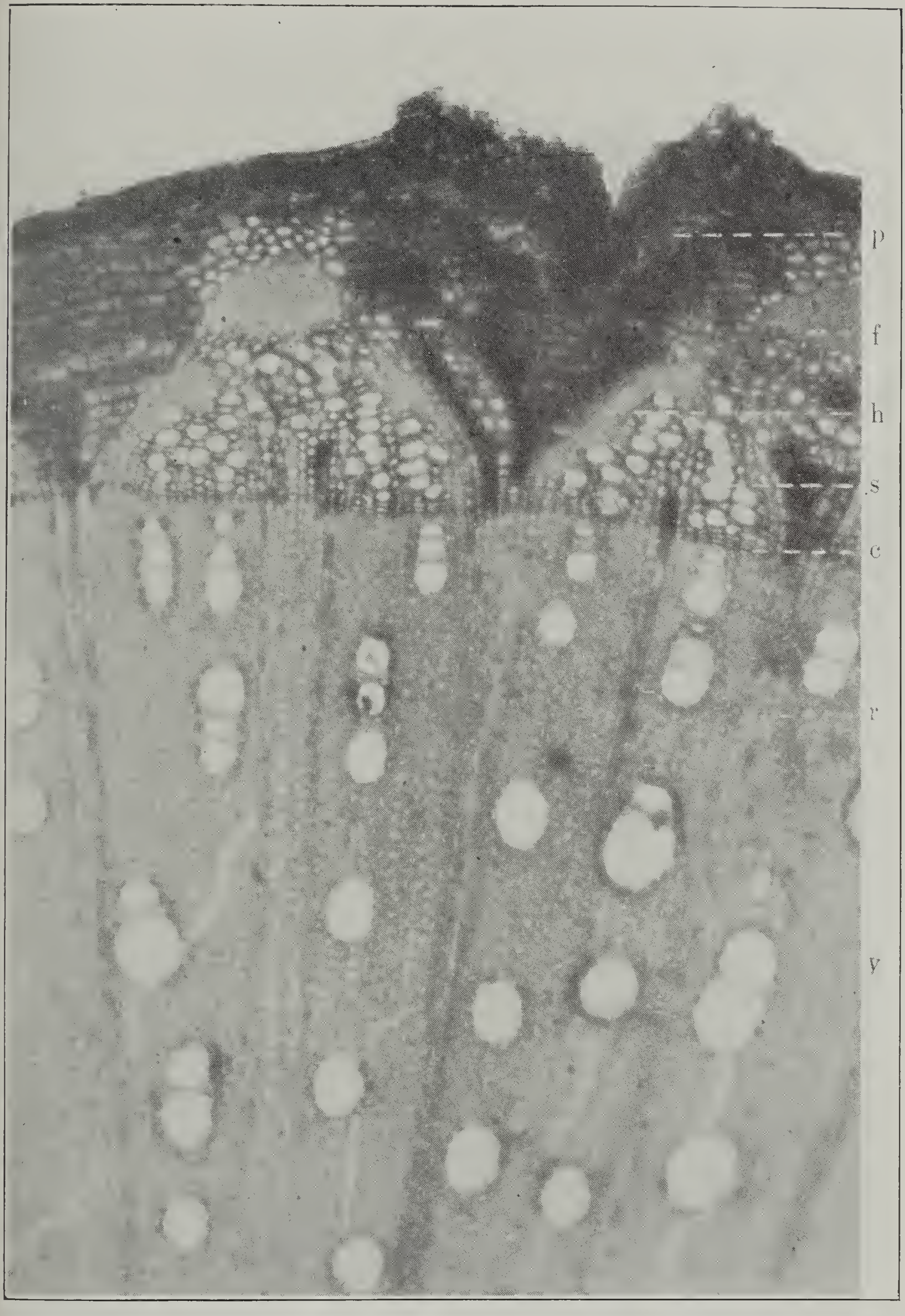

FIG. 4. VITIS ROTUNDIFOLIA, TRANSVERSE SECTION OF BUNDLE, (*p*) PHELLOGEN, (*f*) SCLERENCHYMA FIBERS, (*h*) HARD BAST, (*s*) SOFT BAST, (*c*) CAMBIUM, (*r*) RAY, (*v*) VESSELL.

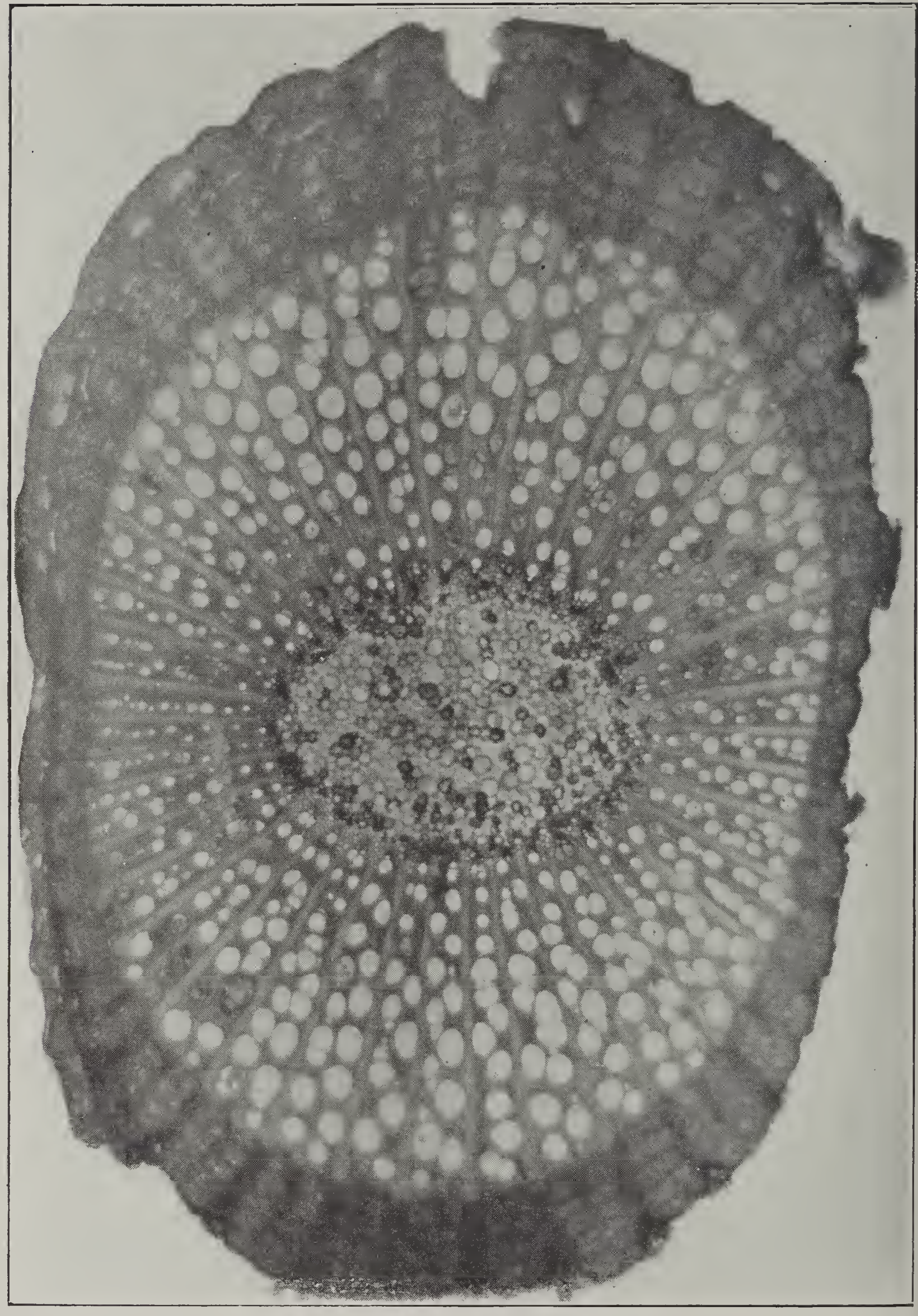

Fig. 5. Vitis Vinifera, Transverse Section of Internode.

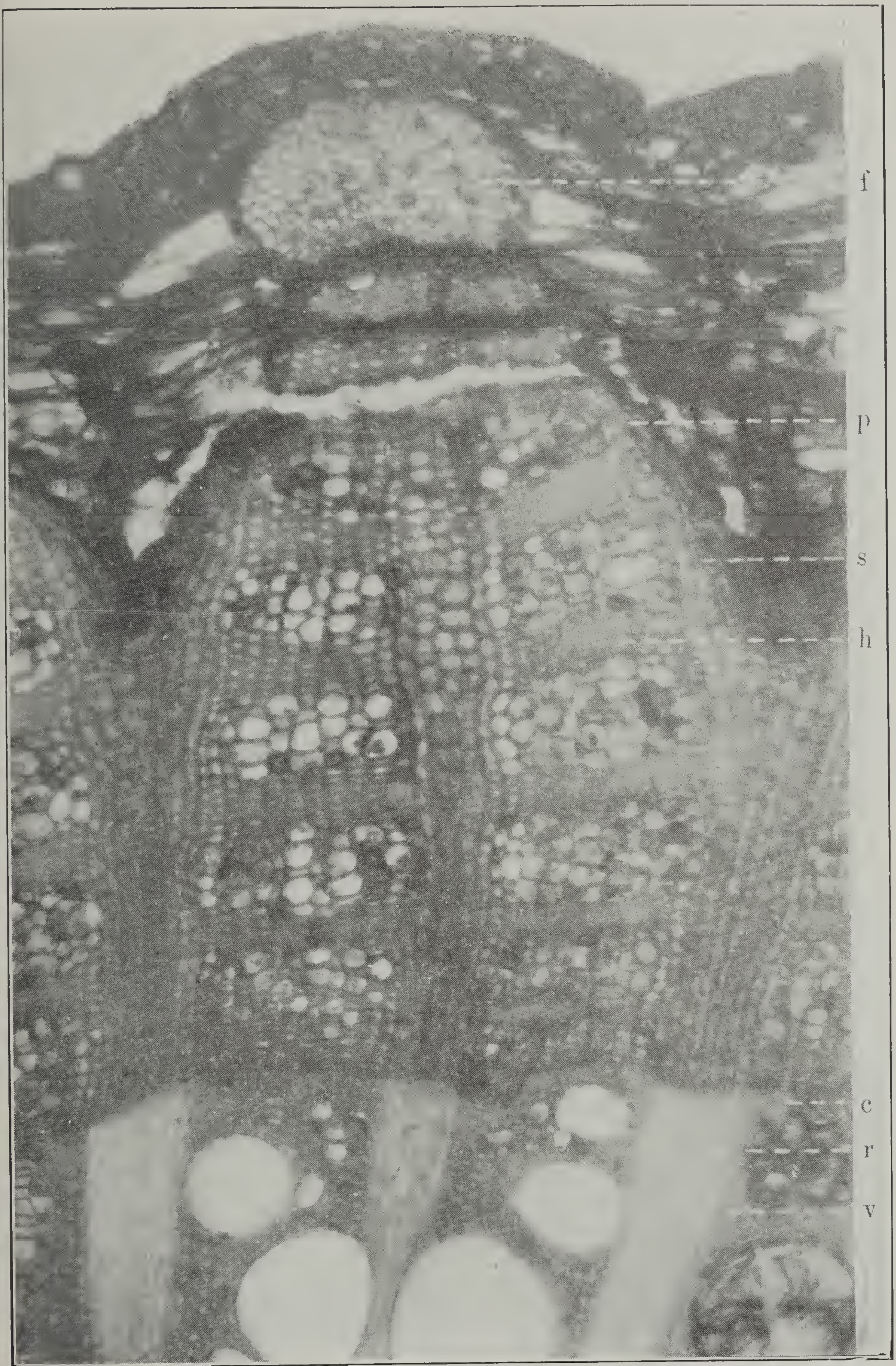

Fig. 6. Vitis Vinifera, Transverse Section of Bundle, (*p*) Phellogen, (*f*) Sclerenchyma Fibers, (*h*) Hard Bast, (*s*) Soft Bast, (*c*) Cambium, (*r*) Ray, (*v*) Vessel.

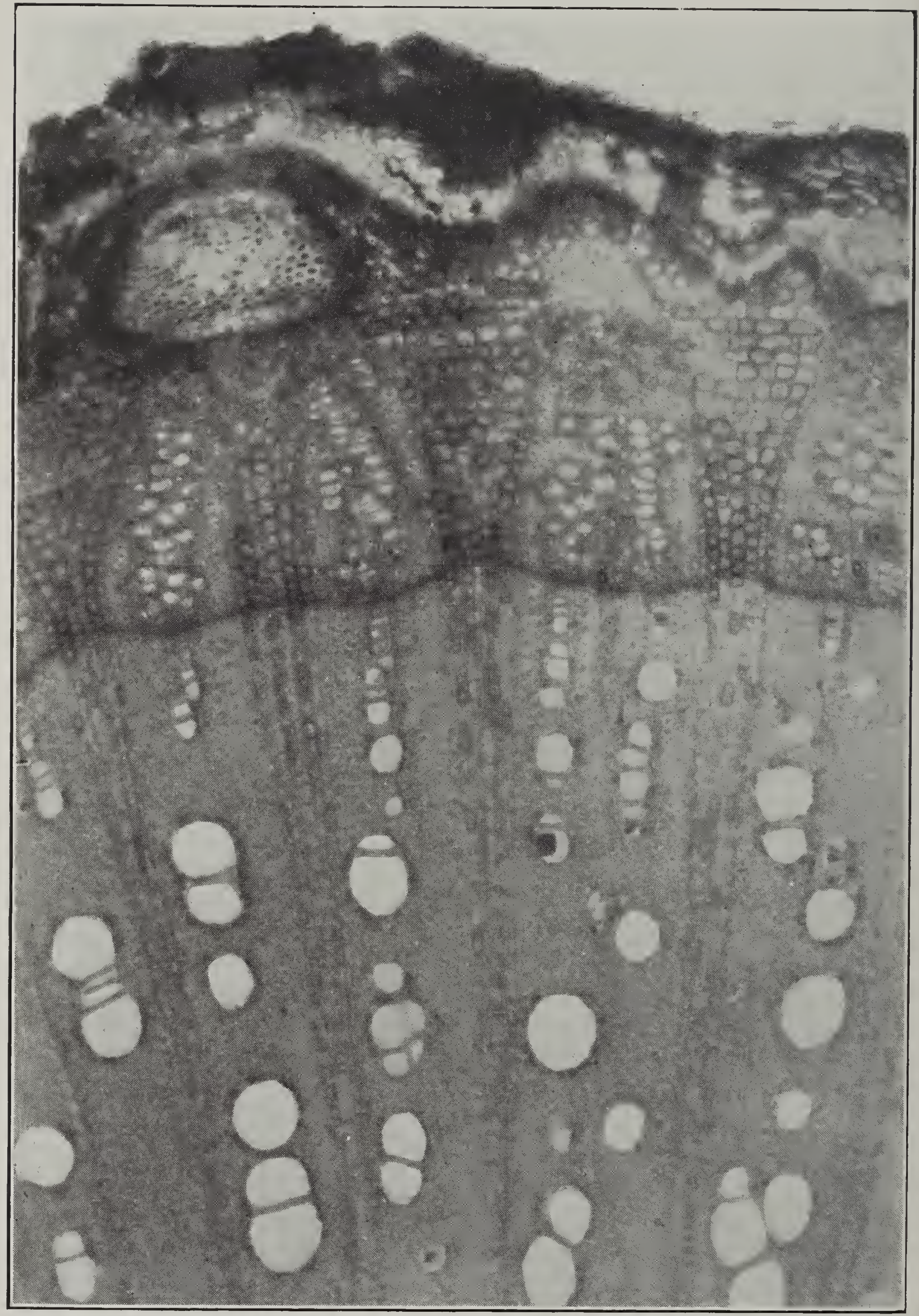

Fig. 7. Hybrid (V. vinifera x V. rotundifolia), Transverse Section of Bundles.

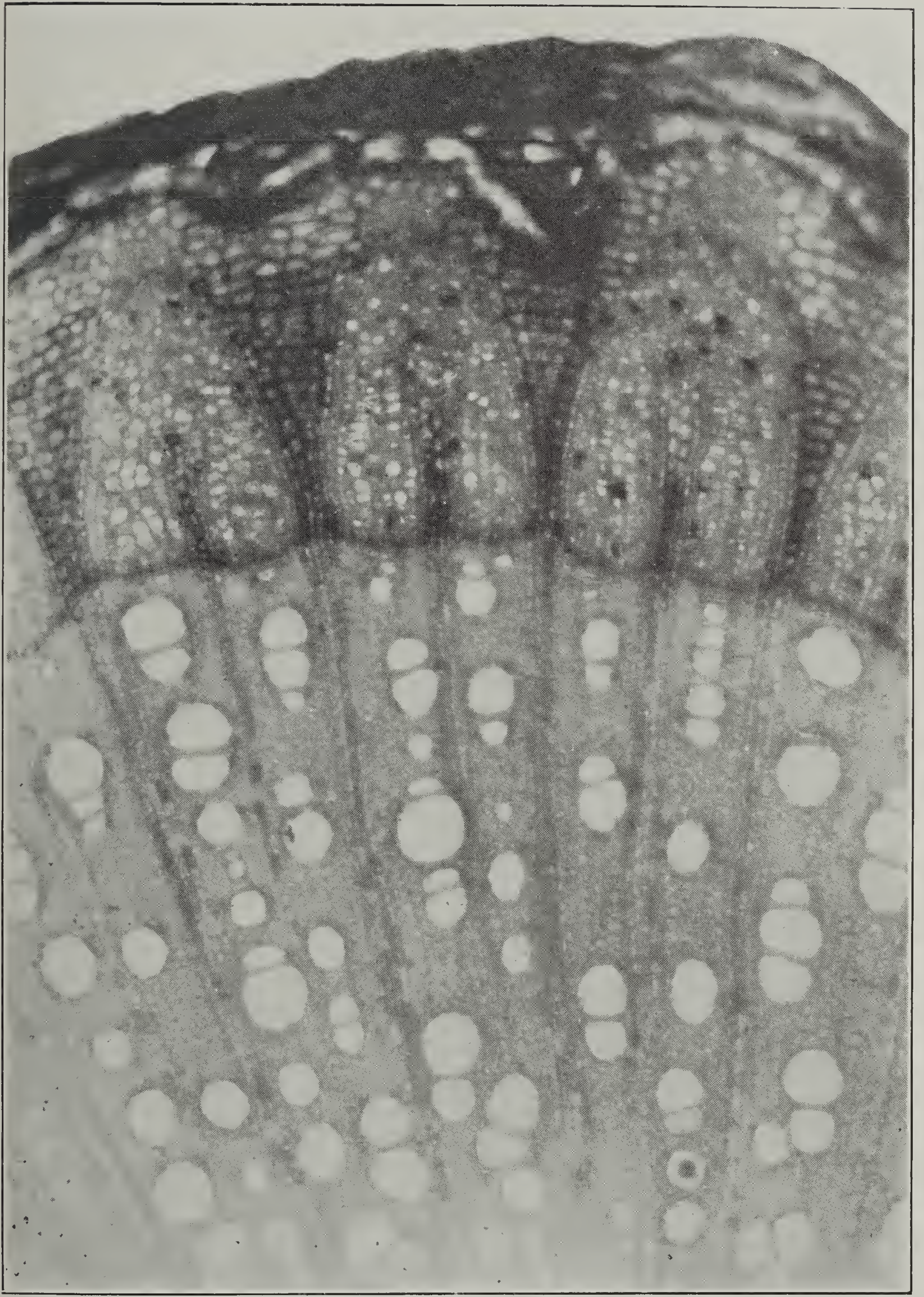

Fig. 8. Hybrid (V. vinifera x V. rotundifolia), Transverse Section of Bundles.

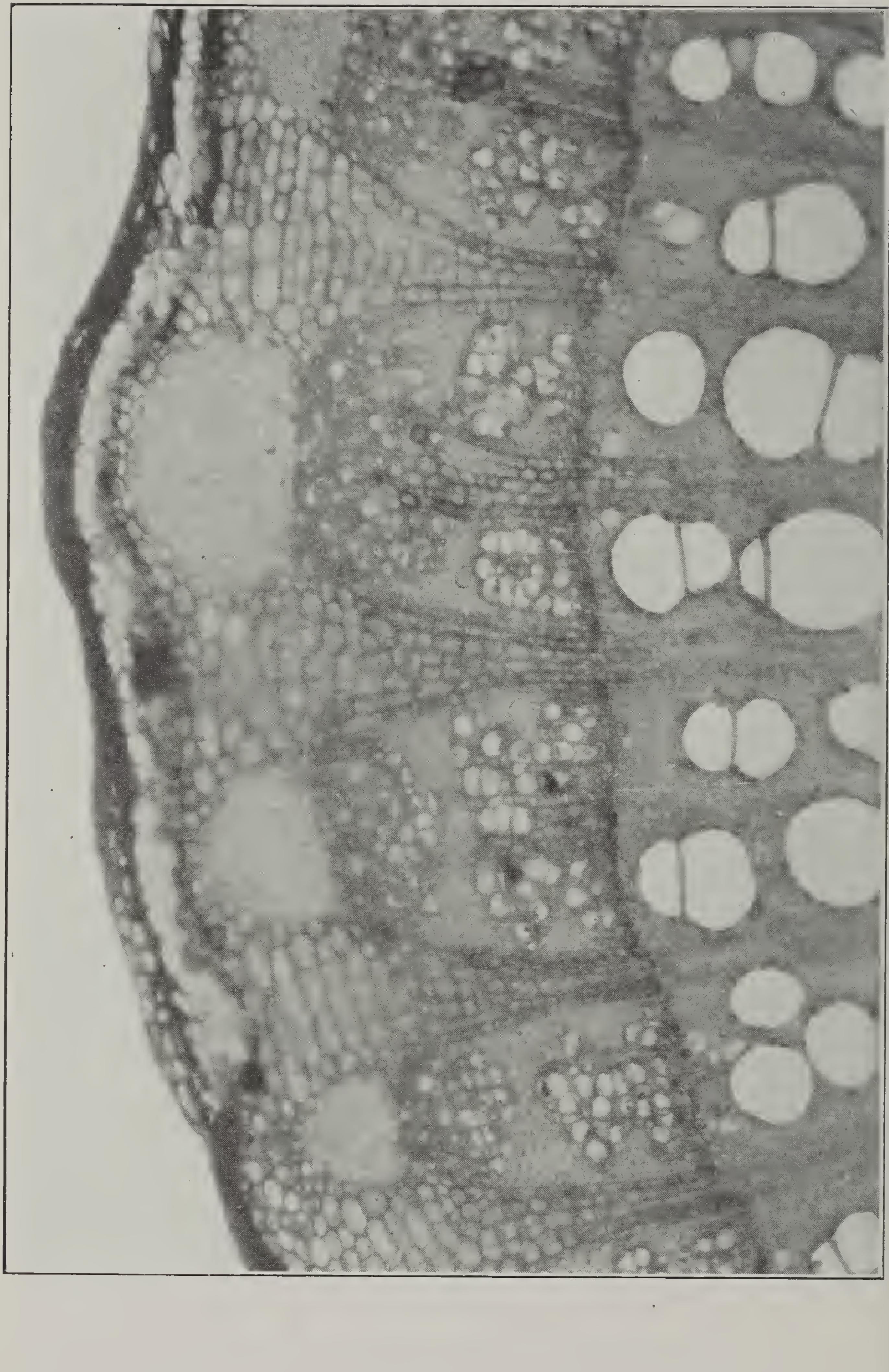

FIG. 9. HYBRID (V. VINIFERA x V. ROTUNDIFOLIA), TRANSVERSE SECTION OF BUNDLES.

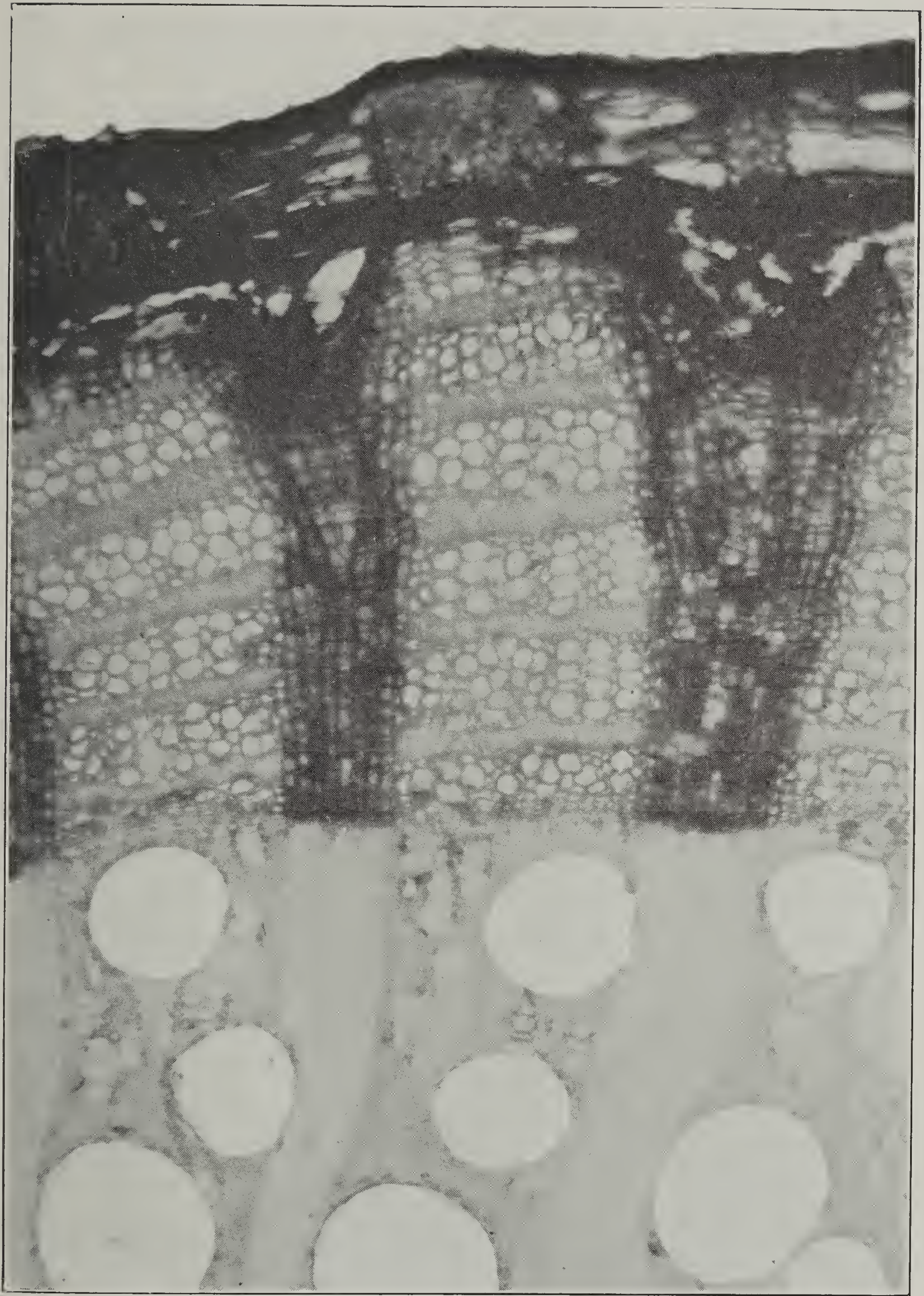

FIG. 10. HYBRID (V. VINIFERA x V. ROTUNDIFOLIA), TRANSVERSE SECTION OF BUNDLES.

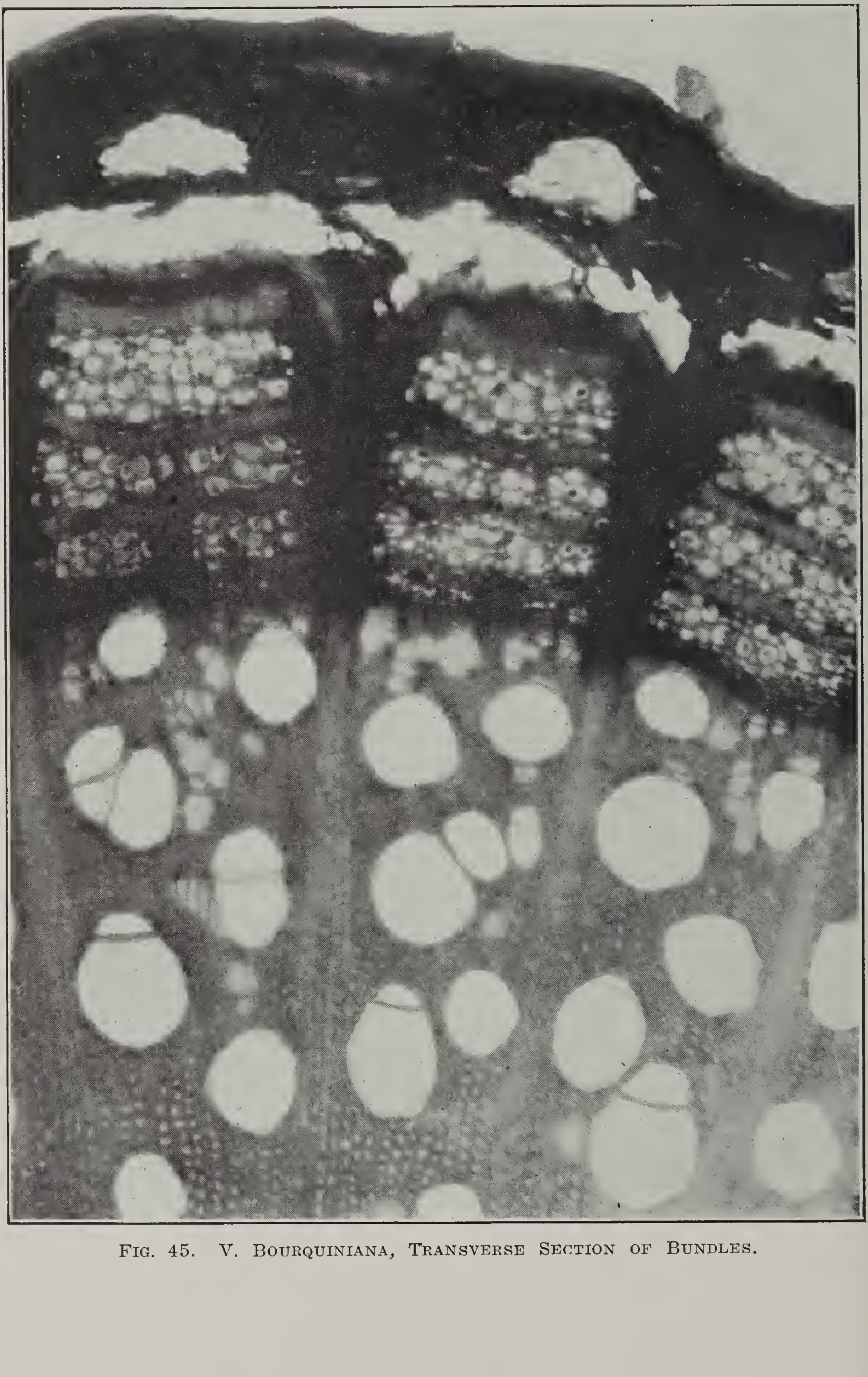

Fig. 45. V. Bourquiniana, Transverse Section of Bundles.

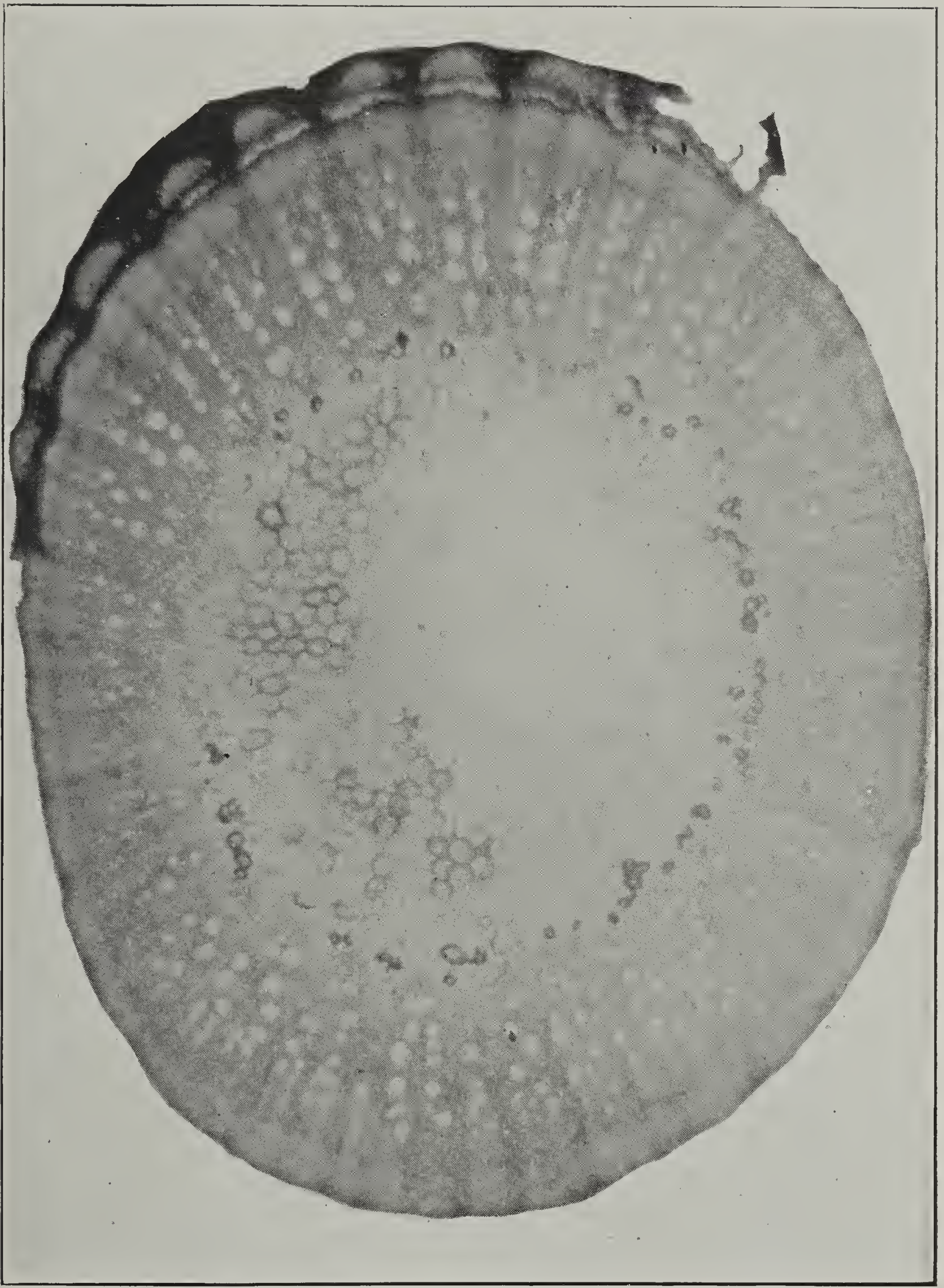

FIG. 46. VARIETY WINCHELL, TRANSVERSE SECTION OF INTERNODE.

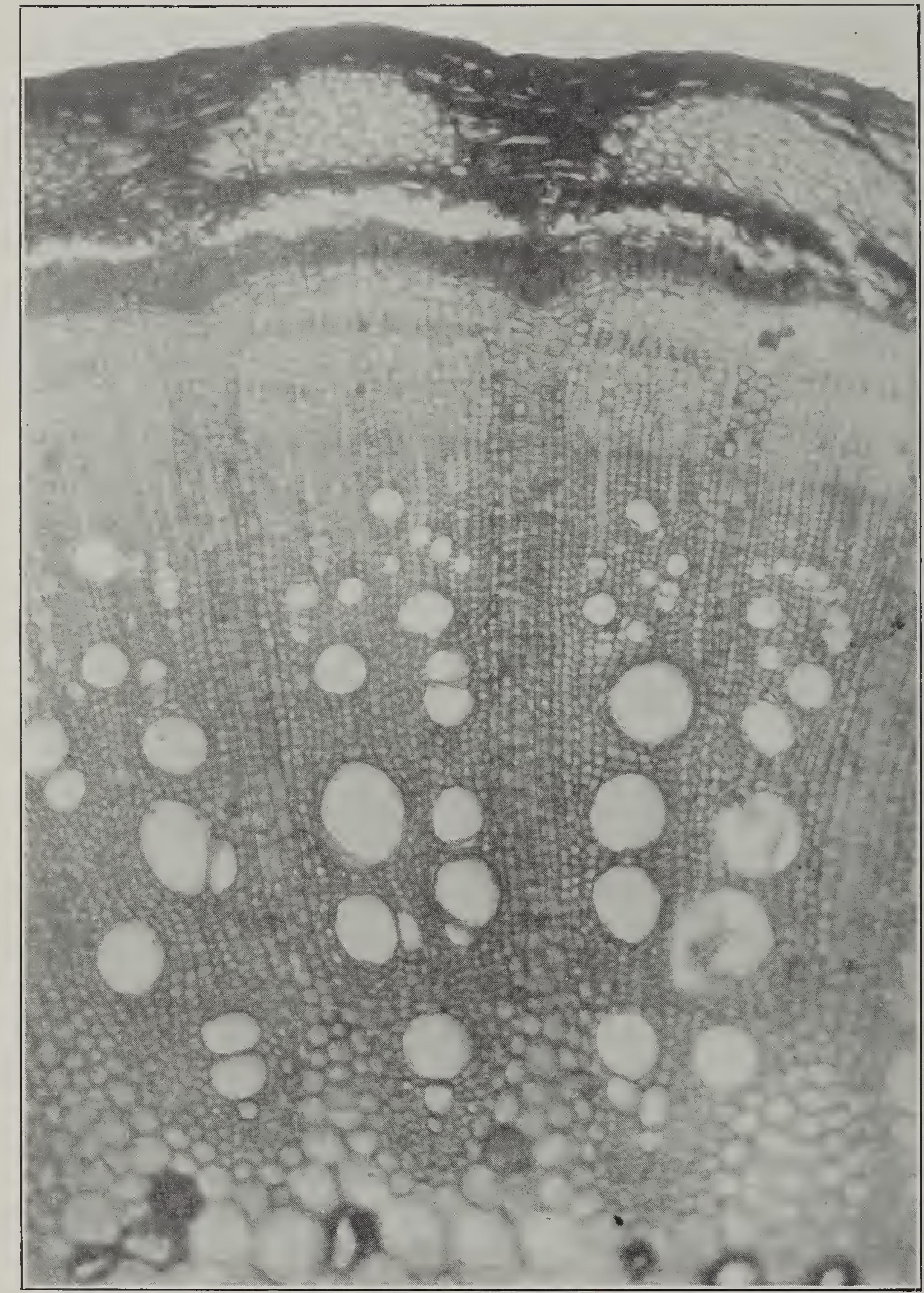

Fig. 47. Variety Winchell, Transverse Section of Bundles.

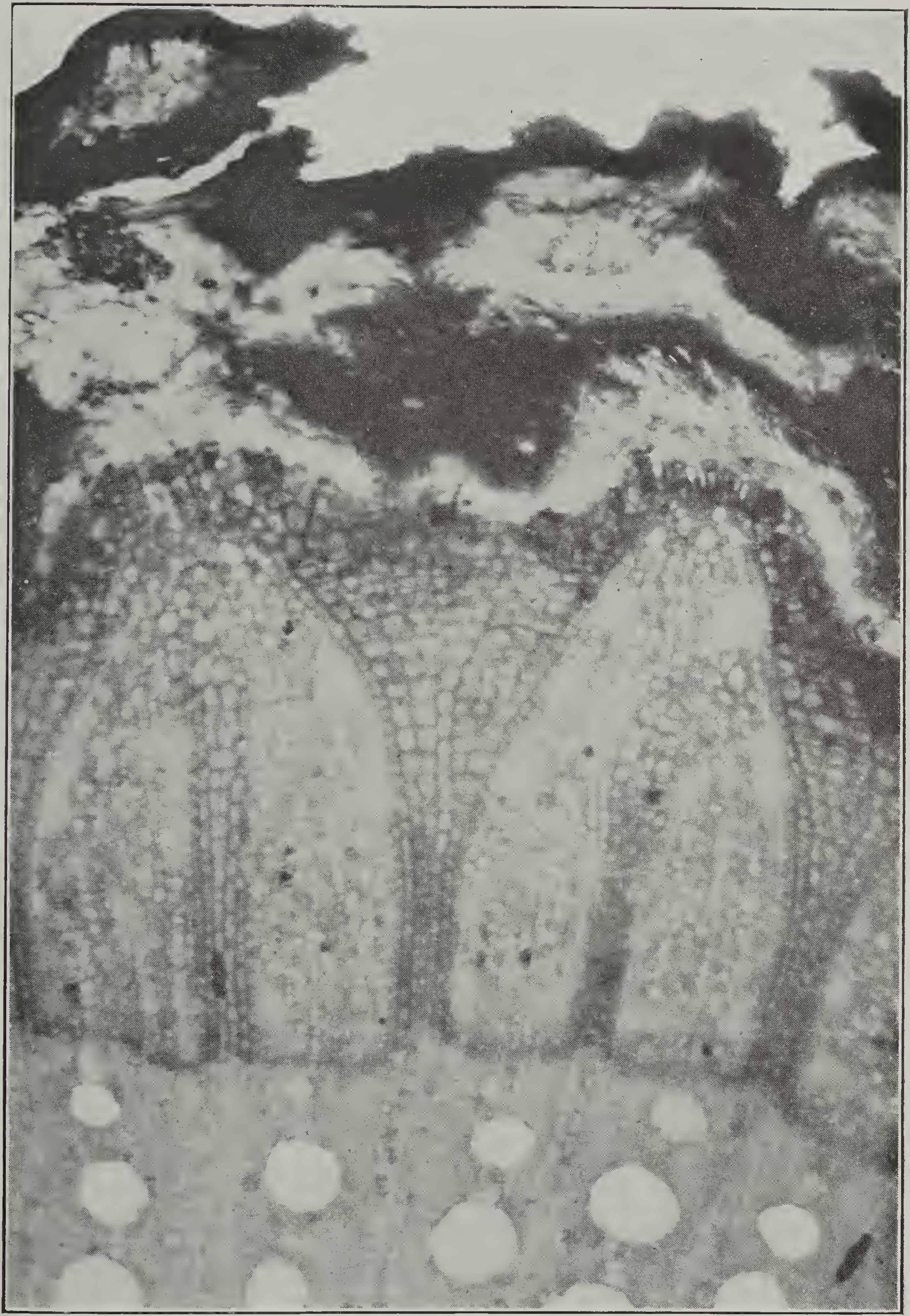

FIG. 76. HYBRID (VAR. WINCHELL x V. ROTUNDIFOLIA), TRANSVERSE SECTION OF BUNDLES.

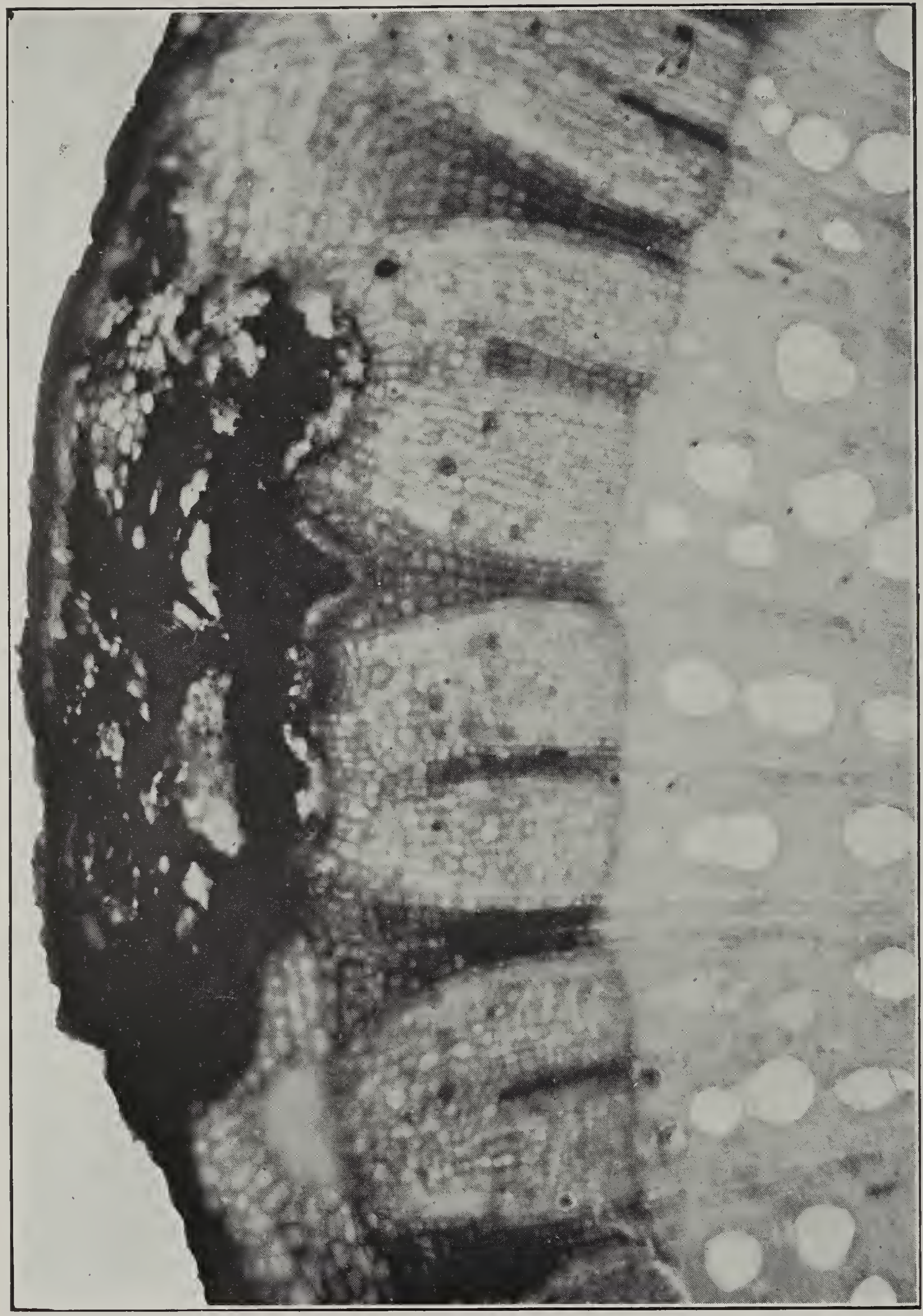

Fig. 77. Hybrid (Var. Winchell x V. rotundifolia), Transverse Section of Bundles.

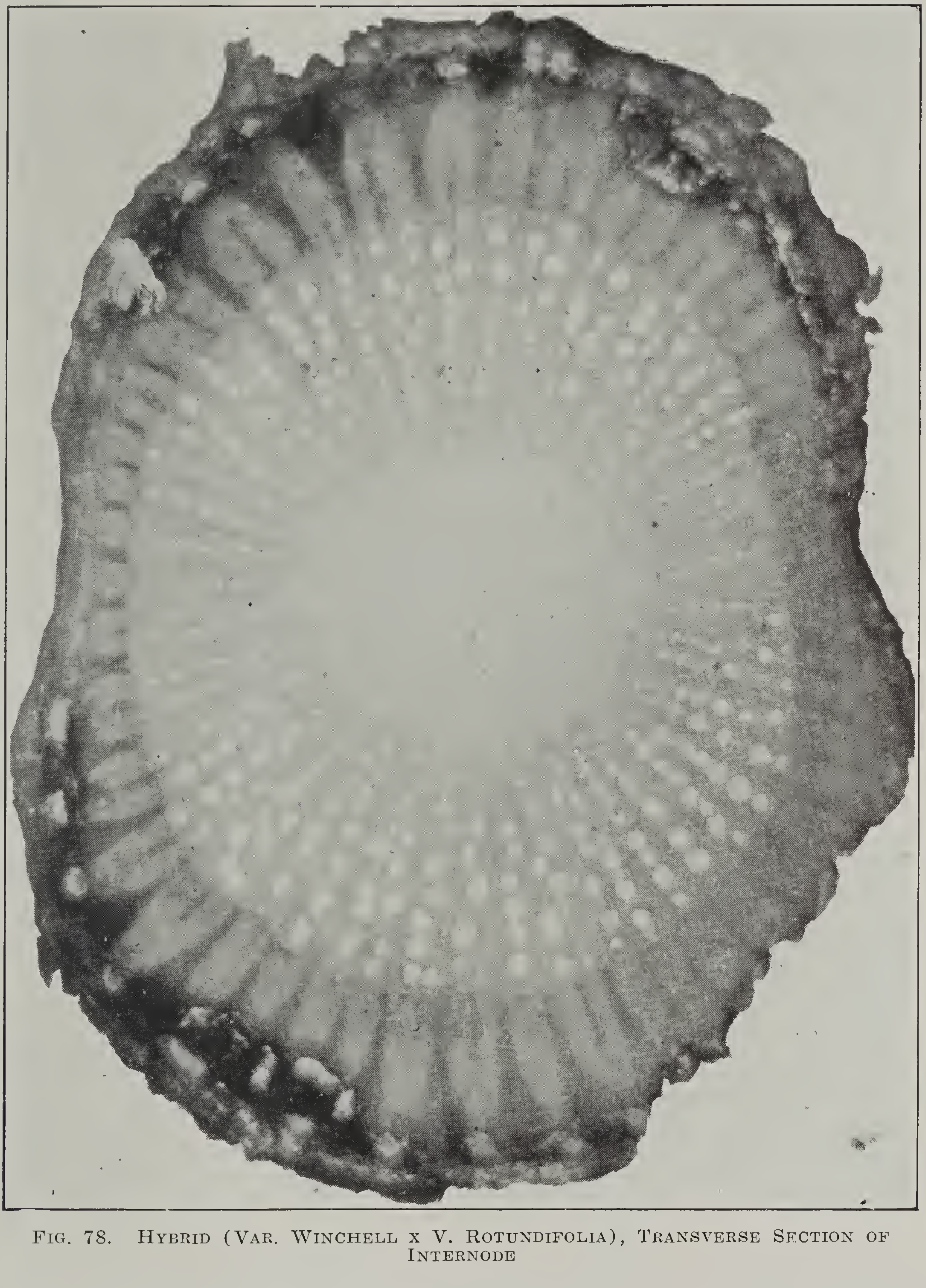

FIG. 78. HYBRID (VAR. WINCHELL x V. ROTUNDIFOLIA), TRANSVERSE SECTION OF INTERNODE

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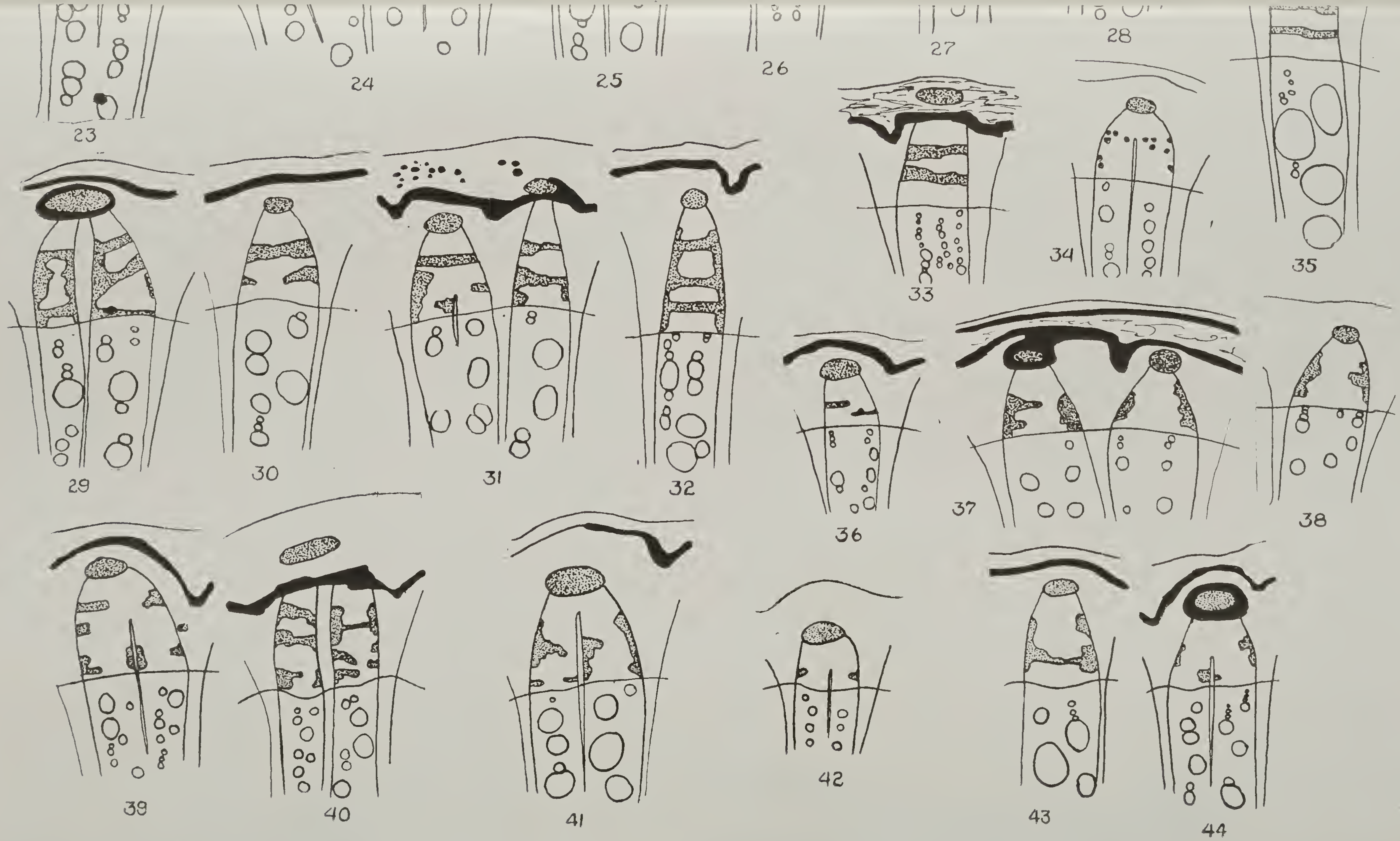
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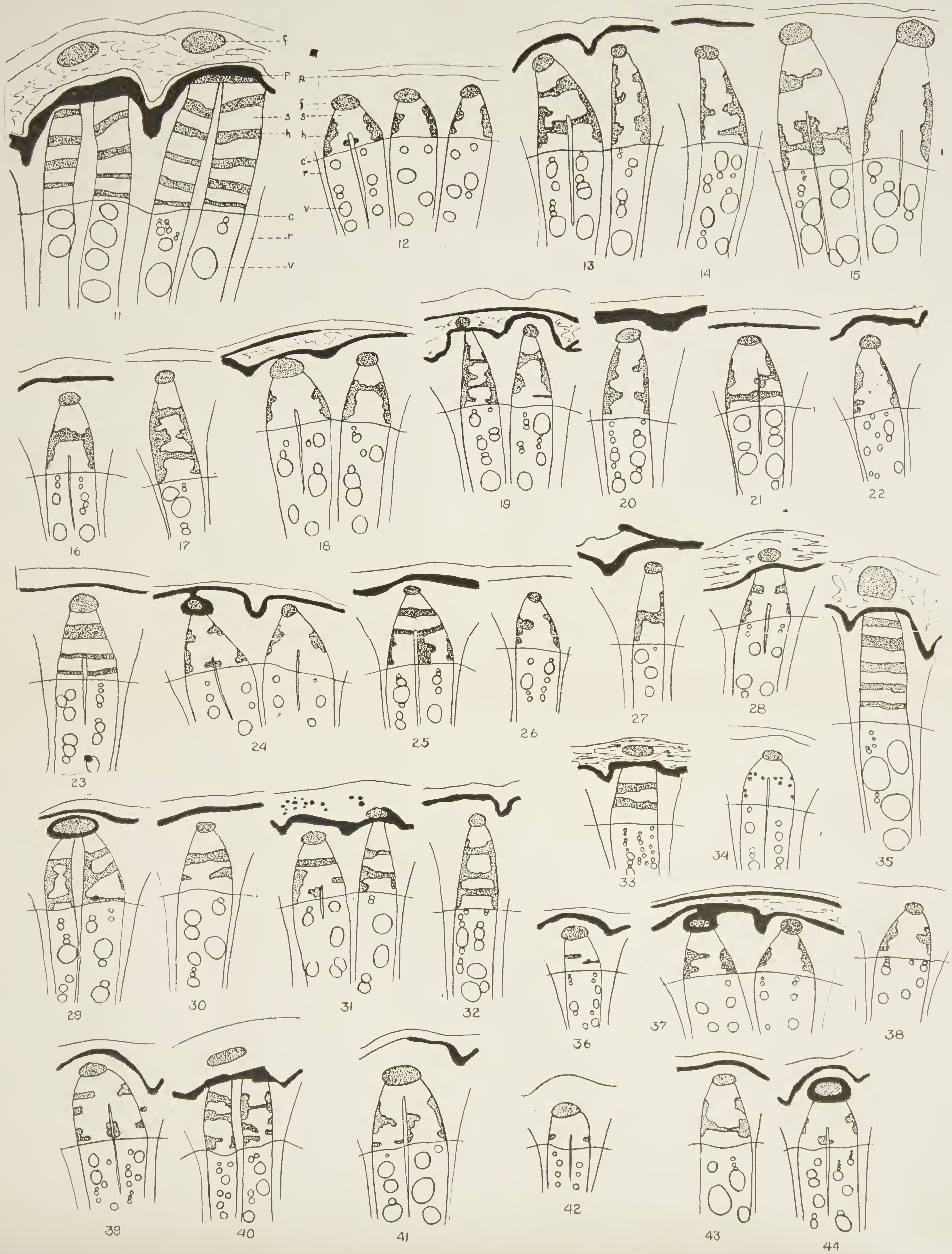
Figs. 11-44. Camera Lucida Diagrams of Transverse Sections of Bundles. Fig. 11. V. vinifera, Fig. 12. V. rotundifolia, Figs. 13-38. Hybrids V. vinifera x V. rotundifolia, Figs. 39-44. Hybrids bourquiniana x V. rotundifolia, (*f*) Sclerenchyma Bundle, (*p*) Phellogen, (*h*) Hard Bast, (*s*) Soft Bast, (*c*) Cambium, (*r*) Ray, (*v*) Vessel.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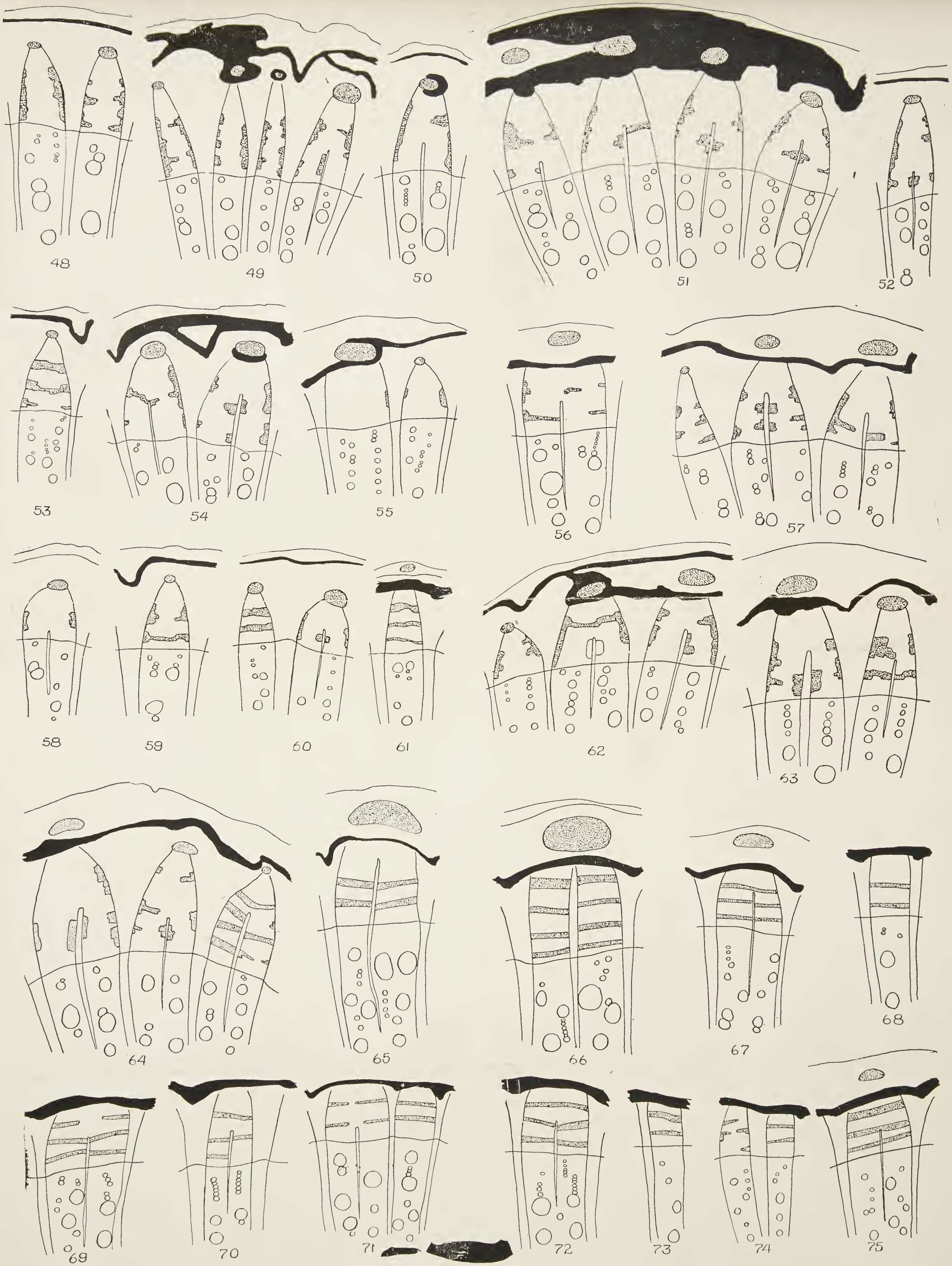
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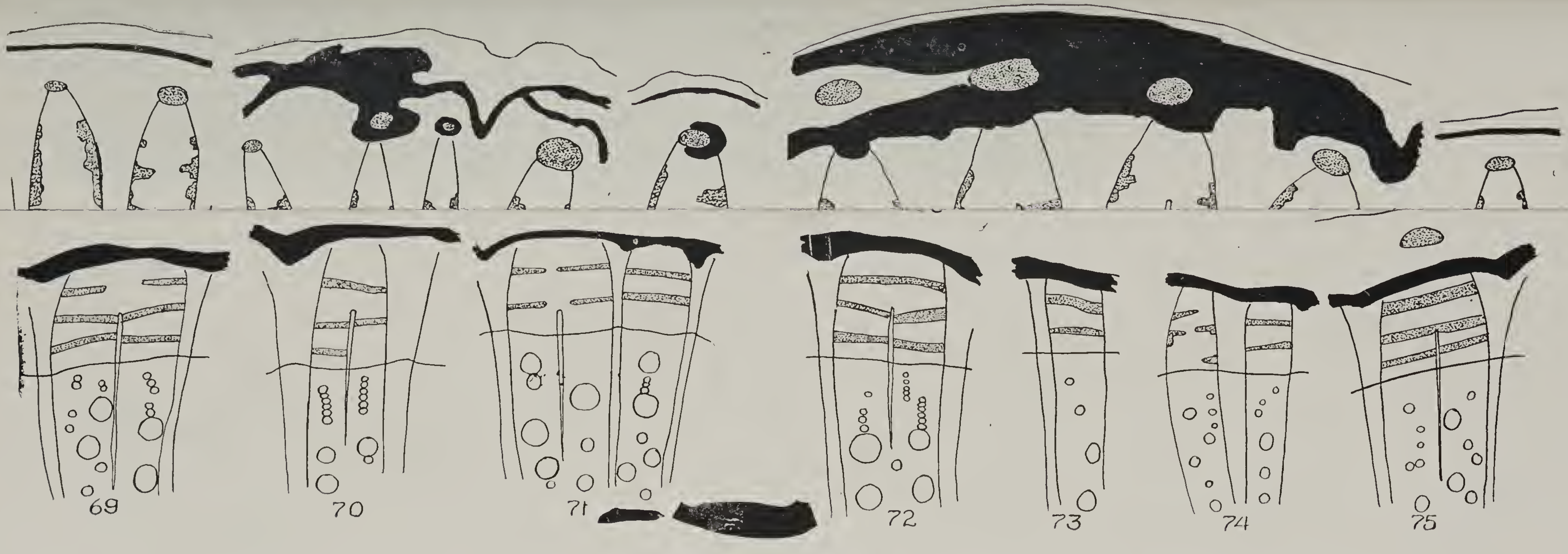
Figs. 48-75. Camera Lucida Diagrams of Hybrids of Winchell x V. rotundifolia.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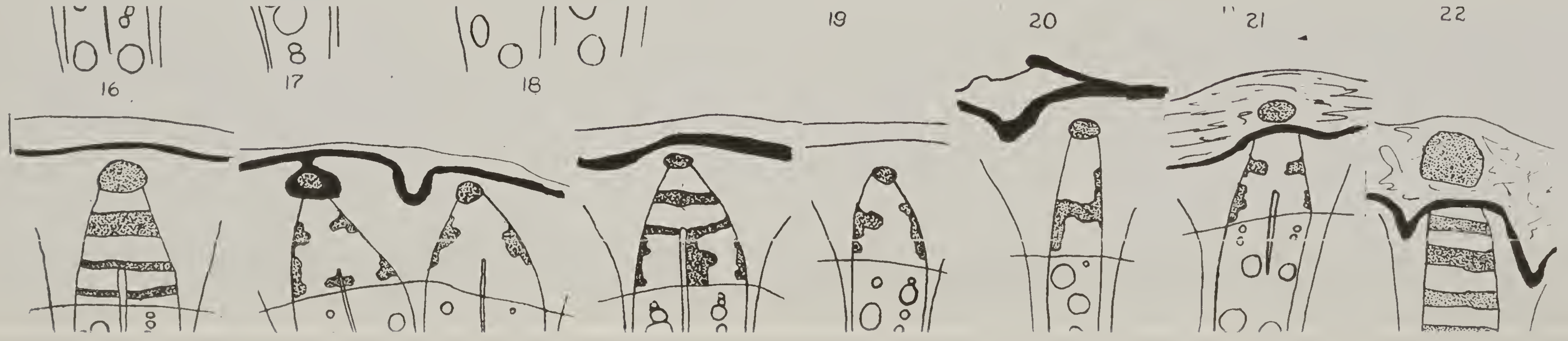
FIGS. 48-75. CAMERA LUCIDA DIAGRAMS OF HYBRIDS OF WINCHELL x V. ROTUNDIFOLIA.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NOVEMBER, 1923 TECHNICAL BULLETIN No. 24

Nitrification and Acidity in the Muck Soils of North Carolina

L. G. WILLIS, Soil Chemist
Division of Agronomy

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
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STATE DEPARTMENT OF AGRICULTURE
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NITRIFICATION AND ACIDITY IN THE MUCK SOILS OF NORTH CAROLINA

By
L. G. WILLIS, Soil Chemist
Division of Agronomy

INTRODUCTION

In Eastern North Carolina large areas of cut-over swamp-land have been drained by means of canals and lateral ditches, and opened up for cultivation thus giving promise of greatly increasing the agricultural resources of the State. In many cases this promise has been fulfilled but on some tracts, the original yield of corn, which has been the crop grown continuously since the soils were cleared, has not been maintained.

Typical of these latter areas are the soils of the Branch Experiment Station Farm at Wenona in Washington county and the soils of a part of the Nissen Farms at Terra Ceia in Beaufort county. On these farms experimental tests of many soil treatments have resulted in the accumulation of data valuable as an aid to a diagnosis of the cause of the unsatisfactory yields of corn.

Both of these farms are located on the level Coastal Plain at an elevation of about fifteen feet above tidewater. The muck deposits are from two to three feet in depth with an underlying subsoil of fine sand, silt and clay. When first cleared, the muck was apparently well drained but in many places there has ensued a gradual settling and compaction with the result that drainage conditions now are very poor. At the Branch Farm, the water-table has not been lowered appreciably by tile drainage, except in the soil directly above the tiles. At Terra Ceia the drainage ditches have failed adequately to drain the unproductive soil beyond the ditch banks.

The practice followed in preparing these lands for cultivation has been fairly uniform over the entire area. Following the extension of the canals, the marketable timber was cut and removed, leaving stumps, fallen trees and other wood wastes to be destroyed. This was partly accomplished by burning, following which corn was planted among the unburned stumps and logs. The early yields of corn were greater than fifty bushels to the acre.

On the more heavily timbered areas the soils were burned over and planted several times in this way. The remaining stumps were then piled and burned so that corn could be planted and cultivated by machinery.

The Nissen farm is fairly representative of the heavily timbered areas, having been reclaimed from forests of "Juniper," *Chamaecyparis thyoides,* Cypress, *Taxodium Distichum,* Gum, *Nyssa sps.*, and other kinds of tree growth, while the soil at the Branch Farm supported, principally, a growth of reeds, *Arundinaria sps.* with but little timber.

As has been stated, the yields of corn were very satisfactory following the first burning. Later experience, however, has shown that many tracts of the cleared soil are incapable of supporting the original high yields. The decrease at the Wenona farm was noticeable after the first year's crop, while on the Nissen farm the yield was maintained for several years.

This difference has, quite logically, been ascribed to differences in the amount of ash furnished by the timber residues. It is probable, however, that the original vegetation on the Branch Farm was limited by deficiency of the soil in plant ash components so that, in the last analysis, the two soils differ in crop-producing power as a result of fundamental differences in composition. Supporting this idea is the fact that now, several years after the last burning, the unlimed soil at the Branch Farm will hardly produce enough corn to harvest, while the soil at Terra Ceia will yield from 20 to 30 bushels to the acre without lime. It would seem, too, that if the plant food components of the ashes supplied by the burning were the sole cause of the original high yields, it should be possible through liming and fertilization to maintain the productivity of the soil. The break-down in the texture of the soil and the resultant retardation of drainage may have been caused by the decay of roots which served as a skeletal support for the deposits when first drained.

Summary of Field Observations

Liming is essential to corn production at the Branch Farm, but fertilization with a mixture of dried blood, acid phosphate and potash salts either alone or with lime has been without effect. At the Nissen farm, liming has only recently become a profitable practice though certain fertilizer treatments have given remarkable results. In no case does it seem that the original high yields have been attained as a consequence of liming or fertilization.

Summarizing the results of experiments on these soils and the experience of men familiar with the district, the following observations are of interest:

1. Application of lime, stable manure, nitrate of soda and kainit, either separately or in combination, increase the yield of corn.
2. Acid phosphate causes a marked depression in the yield.
3. Corn grown on experimental plats on the Nissen farm showed evidence of the accumulation of iron in the nodal tissue, except when grown under treatments producing the highest yields. Otherwise the

amount of the accumulation varied with the treatment and was generally inversely comparable to the yield. (8)

4. Root-rot disease was present on the corn from these test plants, the severity being parallel to the discolorations of the nodal tissue. (8).

The effect of liming on the reaction of soils from plats on the Nissen farm is shown in the following tabulation of results furnished by Dr. J. J. Skinner of the U. S. Department of Agriculture:

TABLE I

THE REACTION OF MUCK SOILS FROM TERRA CEIA AS INFLUENCED BY APPLICATIONS OF LIME AND ACID PHOSPHATE

Treatment	pH
No lime	4.0
1 Ton Limestone per acre	3.9
2 Tons Limestone per acre	3.9
3 Tons Limestone per acre	4.4
4 Tons Limestone per acre	4.5
Acid Phosphate	3.6

DISCUSSION

At first inspection, many of the foregoing observations seem incompatible not only with each other but also with the findings of other investigators.

The reactions noted in Table I show that moderate applications of lime *increased* the acidity of the soil. The same effect is noted as a result of the application of acid phosphate. No other case is on record in which lime has increased soil acidity and Burgess (1) has stated that the long continued use of acid phosphate on an upland soil has resulted in a slight *decrease* in soil acidity.

It is assumed that the work of Hartwell and Pember (6), Conner and Sears (4), Mirasol (12) and others has demonstrated that the ill effect on plants of acid soil conditions is due primarily to the presence in the soil of soluble compounds of iron and aluminum which are toxic. These authors all agree, however, that applications of soluble phosphates decrease the concentration of toxic salts of iron and aluminum.

A plausible explanation of these apparent contradictions is suggested by a consideration of the peculiarities of these muck soils. During the long period of submergence, nitrogen has been accumulated to such an extent that the typical soils now cleared for cultivation contain one per cent or more of that element in organic combination. Up to the time of drainage, this had been protected from oxidation and loss by the excess of water. Subsequently the oxidative bacterial flora may be assumed to have developed so that nitrification of the organic matter would proceed as on normal soils. The heat generated in burning over these soils may have delayed this bacterial development temporarily.

In the process of oxidation of organic matter, if basic material were liberated in amounts equivalent to the nitric acid produced, no change in reaction should have occurred. With a deficiency of strongly basic elements, however, the acidity resulting from the hydrolysis of nitrates of weak bases would increase to the point at which nitrification would be inhibited. This point may never be attained in fact as the nitrate nitrogen is constantly being lost by leaching or through denitrification in the water-logged subsoils.

The reaction of pH 4.0 shown in Table I probably represents the equilibrium reaction in this soil. Obviously, the immediate effect of liming would be to decrease the acidity. This should result in a stimulation of nitrification and the nitric acid produced might quickly neutralize all the lime applied that was not leached from the soil as the bicarbonate. The soil should, thereupon, resume approximately its original reaction showing no residual effect of the added lime.

A similar explanation can account for the increase in acidity following the use of acid phosphate. Fred and Hart (5) have shown that soluble phosphates stimulate bacterial activity in the soil. It is not improbable that the application of available phosphate to these soils accelerates nitrification with the result that the acidity is increased and the yield of corn is depressed.

The toxicity to corn of compounds of iron and aluminum may not be entirely a chemical or physiological problem. Hoffer and Carr (7) have recently shown that the root-rot disease is largely confined to corn plants showing evidence of injury due to soluble iron or aluminum salts. Hopkins (9), too, has furnished data which indicate that soil reaction may be a factor of considerable importance in determining the virulence of one of the organisms associated with corn root-rot. The difficulty experienced in growing corn on these soils may, therefore, be due to the pathogenicity of soil or seed born organisms but it is evident, from the work of the authors last referred to, that the soil reaction and the generation of soluble salts of iron and aluminum are factors of first importance governing the susceptibility of the plants to disease.

It has been stated that nitrate of soda was found very beneficial to corn on these soils. Jones and Shive (10), McCall and Haag (11), and Willis and Carrero (14), have submitted data showing that the nitrate fertilizers, by decreasing the acidity of the culture medium, tend to lessen the absorption of iron by the plant. It is highly improbable that these soils, already high in nitrogen, are in need of nitrogenous fertilizers. A better explanation of the response to applications of nitrate of soda is that it is due to the precipitation of the soluble iron and from the nitrate.

aluminum compounds in the soil by the unassimilated alkaline residue

Potash may be deficient on some of these soils and the effect of kainit can well be due in part, at least, to the actual need of the crop for potassium. Just why the potash should prevent the deposition of iron in the nodal tissues of corn is a question unanswerable until more data shall have been obtained.

The beneficial influence of stable manure, too, seems difficult of explanation unless it be true that the manure serves to inoculate the soil with an ammonifying flora thereby rendering the soil less acid during the early growth of the crop.

Chemical Investigations

For the purpose of studying the fundamental causes of the peculiarities of these muck soils, samples for analysis were taken from the lime-test plats at the Branch Farm at Wenona.

The soils under this test have been cropped continuously to corn for six years, with no planting of other crops. Lime was applied in the form of hydrated lime, ground limestone and marl in amounts equivalent to one, two, three and four tons per acre of calcium carbonate at the beginning of the test and again three years later.

Fertilizer has been applied to each crop at the rate of 300 lbs. of a 3-8-3 mixture composed of dried blood, acid phosphate and potash manure salt. The plan did not include a test of the separate ingredients of this fertilizer mixture so the results for fertilizer treatments represent the average effects of all the fertilizer components whether harmful or beneficial.

The composition of the soil sample from the untreated plat is shown by the following analysis:

TABLE II

COMPOSITION OF MUCK SOILS FROM EXPERIMENTAL PLATS

	Per cent in moisture-free soil
Volatile matter	52.86
Silica	42.69
Ferric oxide	.23
Aluminum oxide	3.67
Phosphoric anhydride	.11
Sulphur trioxide	.36
Calcium oxide	.17
Magnesium oxide	.22
Potash	.45
Nitrogen	1.118
Reaction (pH)	3.62

DISCUSSION

The composition of this typical soil shows that four of its components may be of influence in determining its reaction. The carbon dioxide produced by the oxidation of the organic matter would certainly serve as a solvent aiding in the loss of basic material by leaching. The silica, by tending to form insoluble silicates with these bases, would serve to oppose this action to some extent. With basic material as deficient as it is shown to be in this soil, however, it is probable that the acid silicates would increase, somewhat, the acidity of the soil. Neither carbonic acid nor silicic acid is strong enough to increase the hydrogen-ion content of a soil sufficiently to render the soils toxic to plants. The evidence therefore points to the nitrogen and sulphur components of this soil as the probable sources of acidity.

The oxidation of nitrogeneous organic matter to nitric acid might be expected to develop a high degree of acidity. The oxidation of sulphur compounds with the production of sulphuric acid may share with nitrification the responsibility for this condition, but inasmuch as the content of sulphur is less than that of nitrogen, it is probable that acidity due to sulphuric acid is of less importance than is the acidity due to nitric acid.

Aluminum is present in sufficient amount to combine with all the acid that is likely to be formed by nitrification, and the iron compounds if made soluble would be sufficient in amount to render the soil unproductive for a long period. As compared with other soils of the State, this muck soil seems well supplied with nitrogen, phosphate and potash. Accurate data bearing on the response to fertilizers of crops grown on these plats are insufficient to warrant any definite conclusions being drawn, but if any great benefit or damage should follow the use of a fertilizer it would seem logical, on account of the comparative abundance of nutrient elements in the soil, to ascribe the effect of the added material to some influence other than that of the added plant nutrients.

Effect of Liming on Reaction

Many methods for the determination of the lime requirements of soils have been submitted to test in the hope that some way might be found by which to predetermine the amount of lime necessary to add to a soil to bring it to the optimum reaction for crop growth. Some of these methods have been widely accepted as of considerable value although the discrepancies between the several methods are such as to throw doubt upon the applicability of all of them. These discrepancies have been explained by the assumption that there are several types of acidity in soils and that the different methods vary in the accuracy with which all types are determined.

All methods for the determination of the lime requirement of soils are based on the concept that the effect of liming will be confined to the reaction between the added base and the soil acids. This does not take into account the effect of the change of reaction upon the biological activities of the soil.

In explanation of the anomalous results given in Table I, it was suggested that liming had failed to effect any decrease in acidity for the reason that the addition of lime had stimulated acid production by activating the nitrifying bacteria. The samples from the lime-test plats of the Branch Farm furnish material for a demonstration of the validity of this assumption.

Some results bearing on this point are given in Table III.

TABLE III

The Effect of Liming on the Total Nitrogen Content, Nitrification and Reaction of a Muck Soil

Plat No.	Treatment	Total Nitrogen in Moisture-Free Soil	Nitrate Nitrogen in Moisture-Free Soil		Reaction	
			Original Samples	Incubated Samples	Original Samples	Incubated Samples
		Per Cent	*Parts per Million*	*Parts per Million*	pH	pH
6	None	1.118	35	119	3.62	3.46
13	None	1.121	20	108	3.76	3.63
Average	None	*1.120*	*28*	*114*	*3.67x*	*3.56x*
1	Hydrated lime, 1 ton	1.243	45	151	4.22	3.86
2	Limestone, 1 ton	1.184	35	140	4.18	3.98
4	Marl, 1 ton	1.181	35	135	4.07	3.60
Average	All forms of lime, 1 ton	*1.203*	*38*	*142*	*4.15x*	*3.80x*
5	Hydrated lime, 2 tons	1.178	35	173	4.42	4.26
7	Limestone, 2 tons	1.145	40	184	4.53	4.37
10	Marl, 2 tons	1.129	35	130	4.20	4.14
Average	All forms of lime, 2 tons	*1.151*	*37*	*162*	*4.37x*	*4.24x*
11	Hydrated lime, 3 tons	1.131	35	184	4.91	4.71
12	Limestone, 3 tons	1.125	30	135	5.08	5.03
15	Marl, 3 tons	1.067	40	216	5.14	4.73
Average	All forms of lime, 3 tons	*1.108*	*35*	*178*	*5.02x*	*4.80x*
16	Hydrated lime, 4 tons	1.061	35	216	5.25	4.98
17	Limestone, 4 tons	1.030	35	184	6.01	5.82
19	Marl, 4 tons	1.071	40	216	5.43	5.17
Average	All forms of lime, 4 tons	*1.054*	*37*	*205*	*5.46x*	*5.24x*
9	Fertilizer	1.114	30	59	3.88	3.48
3	Fertilizer—Limestone, 1 ton	1.165	35	151	4.06	3.85
8	Fertilizer—Limestone, 2 tons	1.133	35	130	4.70	4.32
14	Fertilizer—Limestone, 3 tons	1.016	40	189	5.40	4.93
18	Fertilizer—Limestone, 4 tons	1.046	35	173	6.13	5.44

x—Logarithmic averages.

DISCUSSION

It would seem from the analyses in the foregoing table that liming has caused a loss of nitrogen from these soils, the extent of loss being relative to the amounts of lime applied. An alternate hypothesis, that the variations in nitrogen content are caused by differences in the rate of decomposition of the non-nitrogenous organic components of the muck is, however, not impossible.

The unlimed soils are considerably lower in total nitrogen content than are the soils receiving the lighter applications of lime. This may be indirectly the result of the failure of the untreated soils to produce a crop during the last three years of the test.

Liming has decreased the acidity of this soil.

Failure of the original samples to show any consistent differences in their content of nitrate nitrogen suggested that temperature at the time of sampling rather than reaction was the factor limiting nitrification. Portions of each undried sample were, therefore, made up to uniform moisture content, placed into wide-mouthed bottles, and plugged with cotton. After incubation at room temperature and constant moisture content for four months, nitrate nitrogen and acidity were again determined with the result shown also in Table III.

In the incubated soils two facts are clearly demonstrated; (1) that the extent of nitrification, as indicated by the concentration of nitrate nitrogen, is governed by the reaction of the soil, and (2) that incubation increases the acidity. It is probable that this increase in acidity is due to the production of nitric acid by nitrification.

It was evident from inspection of the corn stalks that the presence of soluble compounds of iron contributes to the trouble experienced on this soil. Normally, liming should remedy this condition. Experience has shown, however, that liming at rates up to three tons per acre has not reproduced the original high yields nor has the application of four tons of lime bettered the yield obtained with three tons.

On soils limed sufficiently to produce the maximum crop possible with lime alone, it was found that iron was deposited in the nodes of the corn stalks. It may be, therefore, that the soil at a reaction of pH 5.82, which was the lowest acidity found in the soil of any plat, is sufficiently acid to permit the presence of some soluble compound of iron.

Carr and Brewer (2) have recently published data bearing on this point. They state that aluminum is almost completely precipitated as the hydroxide at pH 5.5, ferric iron is precipitated at about the same point while ferrous iron is completely precipitated at about pH 7.9. Apparently only one of the soils from the lime plats of the Branch Farm has been sufficiently limed to precipitate all of the soluble ferric and aluminum salts.

The reactions shown in Table III may not indicate accurately the condition under which the crops are grown. Much of the nitrogen assimilated by the corn grown on the limed plats must be in the form of calcium nitrate. The rejection by the plants of the basic radical of this salt should tend to maintain a reaction so weakly acid as to protect the plant from the soluble ferric and aluminum compounds.

In soils such as these, high in organic matter and containing excessive amounts of water, the presence of ferrous iron is entirely possible. The four-ton applications of lime have not sufficed to make the soils alkaline enough completely to precipitate ferrous salts as the hydroxide, nor would the calcium bicarbonate rejected by the plant roots effect this result.

The evidence is not conclusive that the unfavorable crop conditions in this soil are due entirely to the state of the surface soil. It has been observed in some pot cultures that applications of lime up to the rate of 15 tons per acre in the surface six inches did not prevent the accumulation of iron in the nodes of corn plants. On removing the soil from the pots, however, it was found that the corn roots had failed to penetrate into the unlimed subsoil, being matted into a dense growth at the junction between the soil and subsoil. Possibly the iron which had accumulated in toxic amounts in the plant was absorbed from the unlimed subsoil rather than from the limed surface soil.

Several investigators (4, 6, 12) have shown that large applications of soluble phosphates aid in the precipitation of these toxic compounds of iron and aluminum and Conner (3) has shown that silicates have an effect similar to that of the phosphates. It is not improbable that the application of large amounts of acid phosphate to these soils would be of considerable benefit especially if used in addition to lime. Silica appears to be present in sufficient amount so that further application would be useless. Extremely heavy applications of lime would be needed to bring this soil to neutrality on account of the potential acidity of the content of nitrogenous organic matter. Such treatments are, of course, impractical and offer no solution to the problems of the farmers of the region. Other remedies must be found which are more rational though less efficient.

There is no doubt that present drainage conditions are the fundamental cause of most of the troubles experienced with these soils. Sherwin, Etheridge and Dunham (13) have reported some of the physical characteristics of a typical muck soil from this section but were able to offer no practical suggestions for facilitating drainage. Until further developments in the solution of this problem, therefore, poor drainage must be accepted as one of the limitations of certain soils of this reclamation district and treatments are to be prescribed on this basis.

Profitable use of these soils may be had by raising crops immune to the toxic influence of acid soils though even these crops may be benefited by liming and fertilization. The blackberry, dewberry and huckleberry (blueberry) should thrive on the poorly drained soils. Rye, too, seems adapted to the soil conditions imposed upon it. If, however, corn is to be raised as it has been in the past, the investigations reported here offer no method for the improvement of the crop yield other than those already suggested by field test; that is, moderate and frequent applications of ground limestone and fertilization with nitrate of soda and a potash salt. Any deficiency of the soil in phosphorus may, perhaps, be remedied by applications of insoluble phosphates without causing the damage which has accompanied the use of acid phosphate.

CONCLUSIONS

1. The typical muck soils of Eastern North Carolina are acid and sooner or later liming will become necessary in order to maintain the yield of corn.
2. Tracts which cannot be adequately drained do not derive the full benefit from liming, probably on account of the presence of soluble salts of iron, especially the nitrate.
3. No method for the determination of the lime requirement of these soils is feasible as the organic nitrogen constitutes a reserve of potential acidity impossible of accurate estimation.
4. The characteristic acid in these soils is nitric acid formed by the bacterial oxidation of the organic matter. The degree of acidity is determined by the reserve of bases in the soil or by conditions which limit the activity of the nitrifying bacteria.
5. Applications of potash salts and nitrate of soda are beneficial and should be given a trial on all soils not giving satisfactory yields.
6. Acid phosphate is injurious when used under present systems of management, and should be applied sparingly or not at all. The ill effect of this fertilizing material may be due to its effect in increasing the acidity by stimulating the growth of the acid-producing organisms of the soil. The insoluble phosphates should be tested on muck soils which do not respond to applications of acid phosphate.

ACKNOWLEDGMENT

Many of the data recorded in this article have been furnished by R. W. Howell, manager of the Nissen Farms, whose well-planned and accurately conducted experiments have been of inestimable help in the orientation of the chemical investigation of the problems involved.

Part of the analytical work reported was done by E. S. Dewar.

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Major Plant Communities
OF
North Carolina

BY

B. W. WELLS

NORTH CAROLINA
AGRICULTURAL EXPERIMENT STATION
Conducted Jointly by the
STATE DEPARTMENT OF AGRICULTURE
and the
NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING
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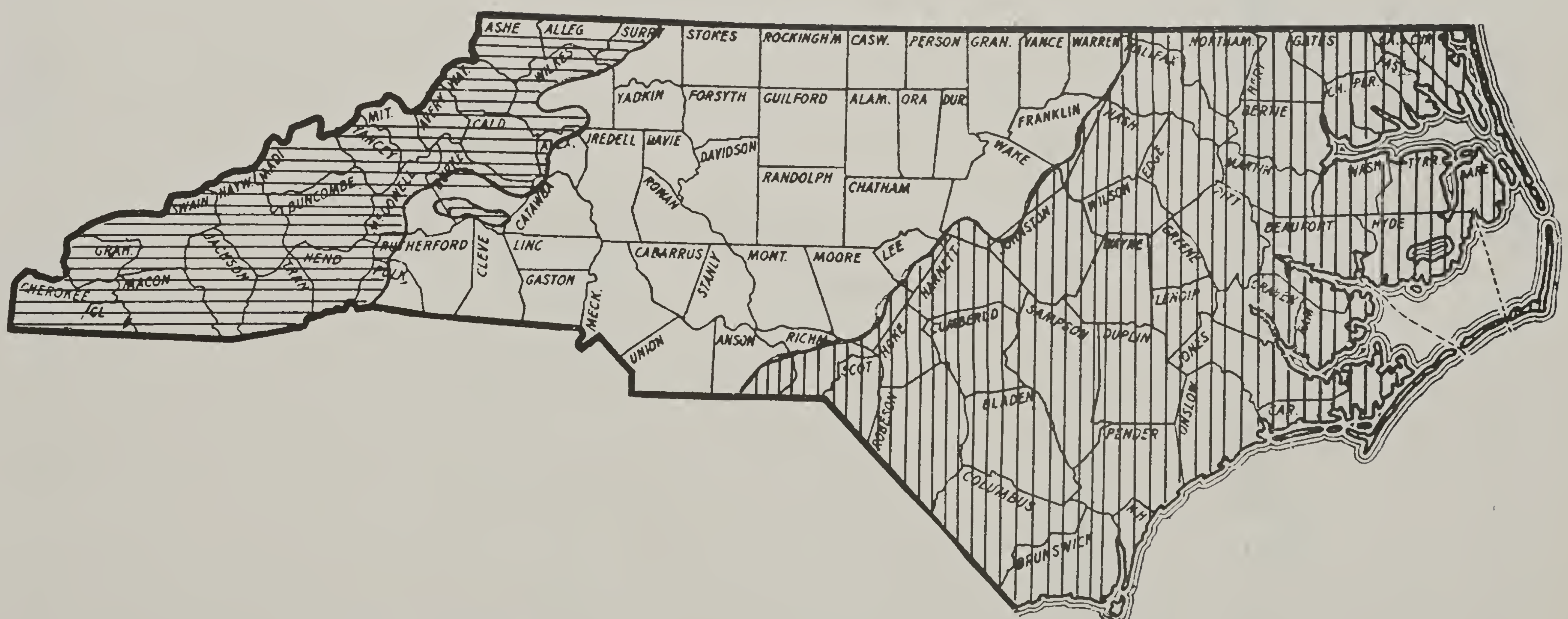
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PAM
ROBESON
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N.H.

Geographical regions of the State: Mountain, Piedmont, and Coastal Plain.

MAJOR PLANT COMMUNITIES OF NORTH CAROLINA

B. W. Wells

In this paper an attempt is made for the first time to present the fundamental plant communities occurring in the geographical area of North Carolina on the basis of a simple modern system of ecological classification.

Only a few states equal North Carolina in ecological interest, for when one considers the climatic range indicated by the balsam fir and and the palmetto and the numerous and remarkable edaphic communities of the Coastal Plain, it may be readily seen that the area of the State is extraordinarily rich in the most striking ecological situations.

To encompass within the space of a single article an outline of the main vegetational features of such an ecologically rich area has meant consistent elimination throughout of all minor data; as indicated in the title, only the outstanding situations under the various topics treated are given.

With regard to habitat considerations, however, it must be stated that brevity here is necessary, more because data based on instrumental research are entirely lacking. However, tentative suggestions are presented which are believed to be of significance in relation to the physiognomic characters delineating the formations.

Ecological study in this State is thus very definitely in the primary descriptive stage. It is to be hoped that the present descriptive paper may make better known the remarkable diversity of conditions in the Southeastern States to the end that investigators of habitat factors may be attracted by the number and variety of the problems found here.

Before presenting the essential part of the paper, it will be desirable to define the important terms used.

DEFINITION OF TERMS

Formation. A major physiognomically distinctive vegetation (dominants) associated with a major distinctive habitat.

Association. A subdivision of the formation characterized by distinctive species dominance which dominance may generally be correlated with minor distinctive physiognomy and minor distinctive habitat.

Climax. The culminating and persisting vegetation (formation or association) in a habitat which is essentially permanent; only a change in climate or diastrophic earth changes of geological time can disturb this community over extensive areas.

Climatic Climax. The climax vegetation on areas characterized by favorable physiographic conditions with regard to plant growth.

Physiographic Climax. The climax vegetation on areas the surface conditions of which are permanently unfavorable to the climatic climax vegetation of the region. Most physiographic climaxes are edaphic climaxes i. e., the soil content is the significant entity in the determination of the vegetation.

Subclimax. A specific formation or association which is changing very slowly due to the relative stability of its habitat. This stability, however, does not possess the permanence which characterizes the climax habitats of geological time periods.

In the use of names of dominants for the formation names, it is not to be implied that these plants are present in all associations of the formation or in equivalent formations in other parts of the world. As types they illustrate the physiognomy of the formations.

The undesirability of using plant names for the major groups is recognized, yet in the present state of ecological progress, it is probably best. Students in the future may easily make the necessary correlation by pointing out the ecological synonomy existing between the different communities.

In the organization which follows, no account has been taken of the minor plant formations such as those of the lichen and moss. With regard to the communities treated, only the formations and their principal associations are outlined in this paper.

With regard to nomenclature, Small's "Flora of the Southeastern United States" has been followed.

FORMATIONS

1. Uniola-Cakile formation
2. Juncus-Spartina formation
3. Typha Scirpus formation
4. Potomogeton-Nymphæa formation
5. Ilex-Myrica formation
6. Campulosus-Sarracenia formation
7. Nyssa-Taxodium formation
8. Quercus-Aristida formation
9. Syntherisma-Erigeron-Andropogon formation
10. Quercus-Acer-Pinus formation
11. Abies-Picea formation

1. **Uniola-Cakile Formation** *(Uniola paniculata, Cakile spp.)* Dunes.

Distribution. On seaside dunes and along sandy shores of sounds and rivers near the sea. Its best development is on the southeastward facing coast south of Cape Hatteras. Long stretches of the northeastward facing coast north of Hatteras are not characterized by the line of low dunes which form the typical habitat of the Uniola grass.

Habitat. The well-drained, humus-free soil of the dunes plus the omnipresent wind of this coastal locality make this habitat a xerophytic one, despite the condition of abundant rainfall.

Physiognomy. The well-known xerophytic structure of seaside dune plants is observed here.

Associations. In addition to the associations dominated by the type species, the following are often locally prominent:

Spartina patens
Oenothera humifusa
Croton punctatus

Successional Relations. Physiographic changes may locally affect the formation but on the relatively stable dunes of the southeastward facing shore, the formation maintains itself throughout long periods of time so that we may here speak of this major community as a physiographic subclimax formation. Eventually, as indicated by the older dunes lying back from the sea, this formation is succeeded by the maritime Quercus-Juniperus association of the Quercus-Acer-Pinus formation.

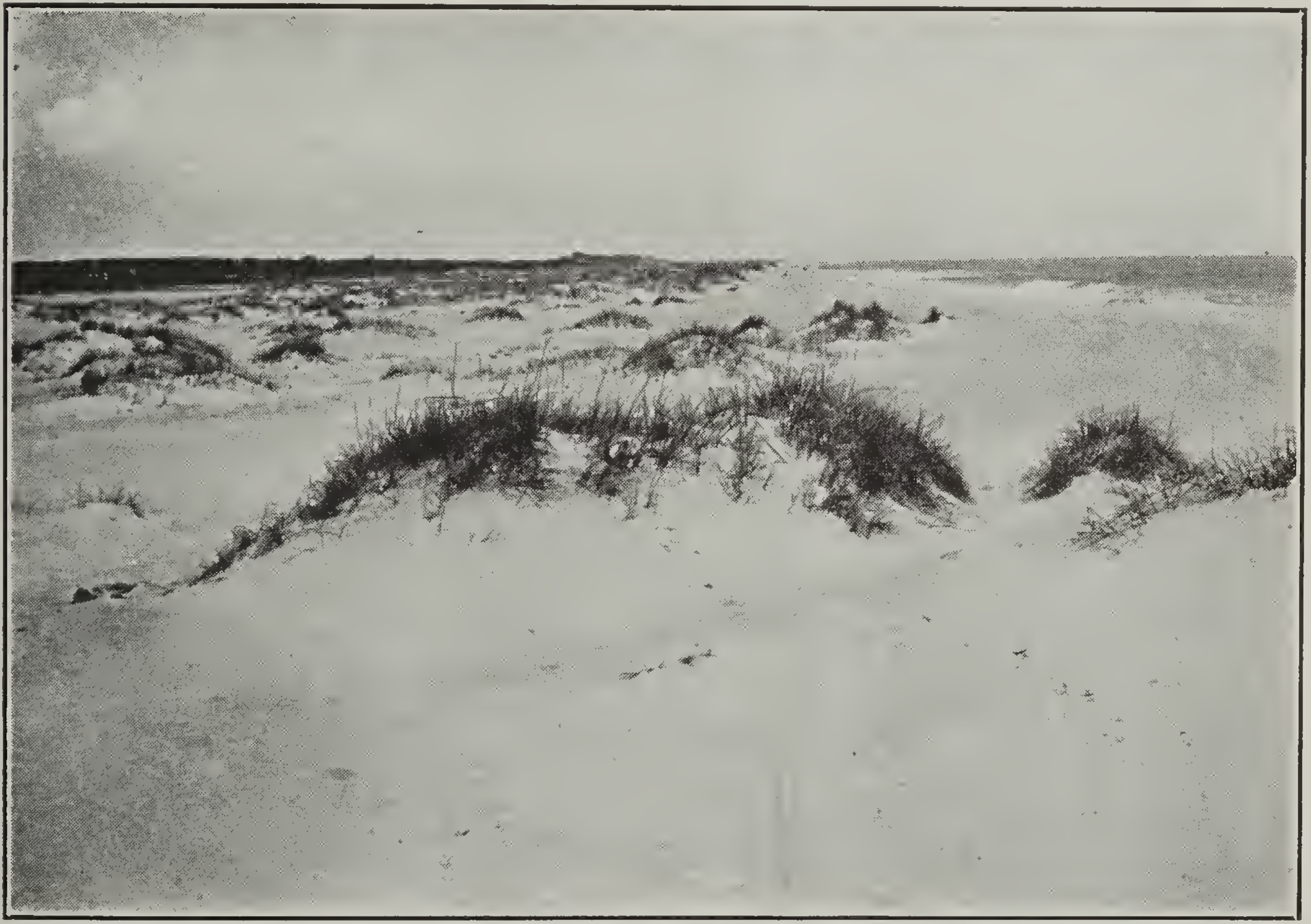

Fig. 1. Uniola-Cakile formation on Coastal dunes near Wilmington, N. C.

2. **Juncus-Spartina** *(Juncus roemerianus, Spartina stricta).* Salt Marsh.

Distribution. Along the salt-water sound shores and borders of brackish river mouths.

Habitat. The salt marshes; substratum of dark silty material saturated with salt water.

PHYSIOGNOMY. The well recognized xerophytic adaptations.

ASSOCIATIONS. Some of the related associations are:

Phyla nodiflora-Monniera monniera (Dune marshes).
Sesuvium maritimum-Tissa marina (Tidal flats).

SUCCESSIONAL RELATIONS. The habitat occupied by this formation is in general changing in the direction of mesophytism or a different type of xerophytism through the physiographic process of aggradation. On the seaward side of the sounds, the Uniola-Cakile community may replace it while on the mainland side, the Ilex-Myrica complex or some association of the mesophytic forest formation.

No discussion can be given here of the striking successional phenomena existing between the associations of the formation.

3. **Typha-Scirpus Formation** *(Typha latifolia, Scirpus spp.)* Fresh Water Marsh.

DISTRIBUTION. Locally throughout the State.

HABITAT. Fresh water marshes along borders of fresh water sounds, lakes and slow streams.

PHYSIOGNOMY. Hydrophytic plants mostly with well developed aeriferous tissues; no marked development of transpiration inhibiting devices.

ASSOCIATIONS. Some of the outstanding associations in this formation, other than those involving the dominants are:

Proserpinaca palustris
Juncus effusus
Zizaniopsis miliacea
Sacciolepis gibba

SUCCESSIONAL RELATIONS. On the borders of the few and restricted artificial areas of water occurring in the Piedmont and montane sections of the State, the successions are similar to the well-known northern situations. In the eastern section, this formation may be succeeded by the Ilex-Myrica community, various associations of the eastern mesophytic forest and occasionally by the Nyssa-Taxodium swamp forest.

4. **Potomogeton-Nymphaea** *(Potomogeton pectinatus, Nymphæa odorata).* Shallow, open water community.

DISTRIBUTION. Locally throughout the State, but chiefly in the quiet waters of the eastern section.

HABITAT. Completely under water or leaves floating on the surface.

PHYSIOGNOMY. The familiar adaptations to the aquatic habitat.

ASSOCIATIONS. Among the prominent associations in this formation are:

Potomogeton spp.	*Chara spp.*
Utricularia spp.	*Zostera marina*
	Pontederia cordata

SUCCESSIONAL RELATIONS. The familiar migration of shore formations accompanying aggradational progress.

5. **Ilex-Myrica** *(Ilex glabra-Myrica cerifera).* Pocosin or Bay Land.

DISTRIBUTION. Locally throughout the lower or eastern half of the Coastal Plain.

HABITAT. Non-draining, humus-filled, sandy, saturated soils chiefly, but wet muck and peat soils also carry this formation. Free surface water of 3-9 inches depth commonly present.

PHYSIOGNOMY. Evergreen shrubs chiefly of the near sclerophyll type. Definite xerophytic tendencies uniformly evident in leaf structure.

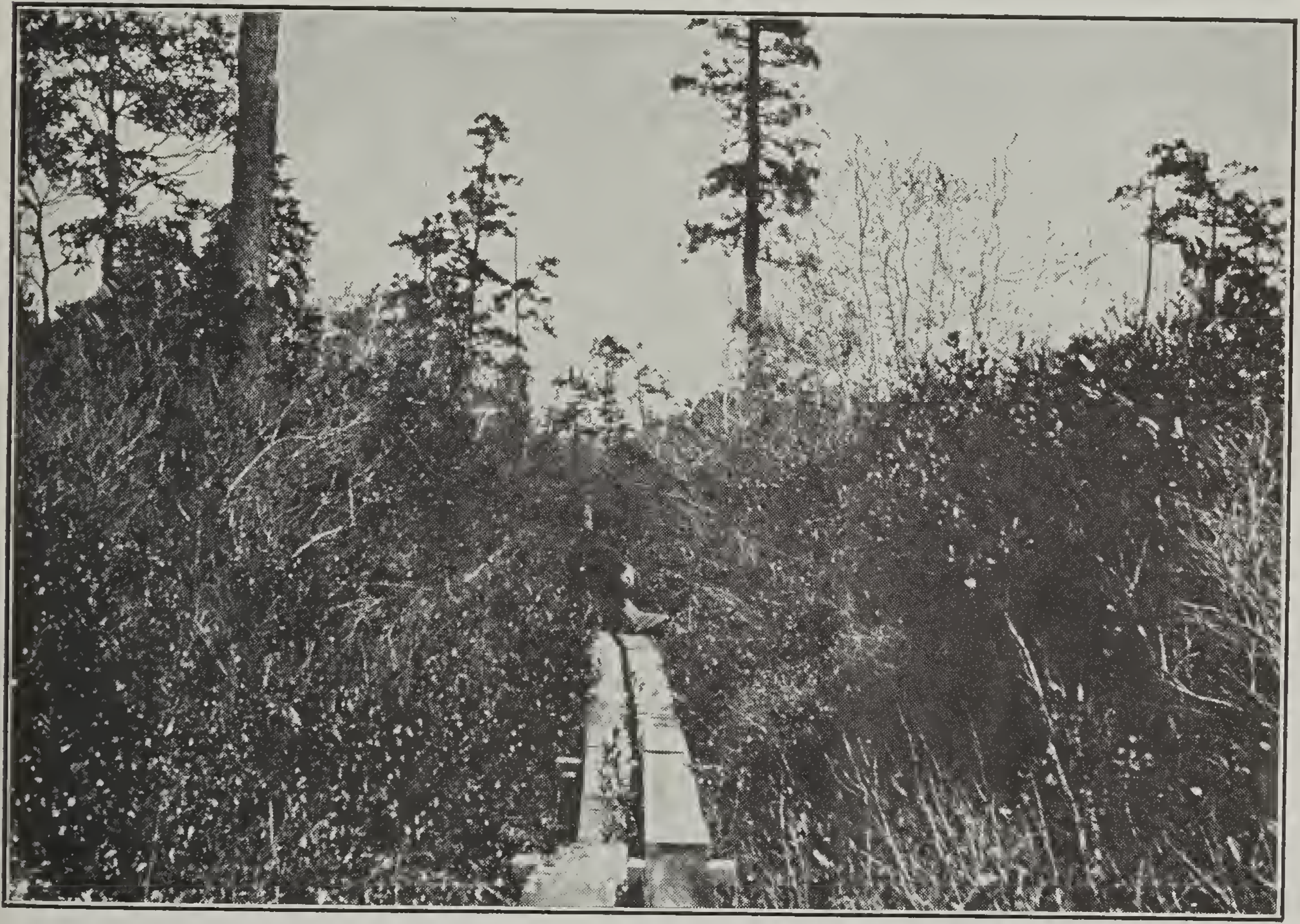

FIG. 2. Ilex-Myrica Formation

ASSOCIATIONS. In addition to the two dominants given, some common associations are dominated by

Cyrilla racemosa	*Baccharis halimifolia*
Pieris nitida—Leucothoe elongata	*Arundinaria arundinaria*
	Vaccinium spp.

This formation merges in many places into other formations; its boundaries locally are not usually very sharp. So prominently, however, does it stand out over large areas, that it is believed best to give it formational rank.

There can be little doubt that the xerophytism evinced in the Ilex-Myrica formation is homologous to the xerophytism of the northern bog plants. Nothing in the way of technical investigation of the soil factors operating in them has yet been prosecuted, but one thing is certain, viz: that the factor of low temperatures which is so characteristic of northern bogs may be ruled out. This leaves the soil toxin theory as furnishing the most probable explanation of the physiological dryness of this habitat.

One very striking fact regarding the distribution of Myrica within the formation has been observed. This plant disappears where the sand disappears from the soil, i. e., in muck and peat soils, Myrica is almost certain to be absent.

Though present in the small openings in this formation, Sphagnum never succeeds beyond a very minor role; the climatic growth conditions are too favorable for the development of the shade-producing vegetation, hence the inability of Sphagnum to become even a local dominant.

Successional Relations. Space will not permit an analysis of the intra-formation successions.

In upland semi-swamp areas, where the pine association has been removed, this community is almost certain to come in and maintain itself even in the presence of frequent fires. Pines (especially P. serotina) will almost always be found scattered through it, but in its maximum development, these are never dominant.

Once this formation has assumed a full control of an area, it may persist for a sufficiently long period of time to make it a sub-climax. The deep shade it produces tends to prevent the pines from appearing in it. Under conditions of frequent fire, the community is quickly restored from the underground stem structures. If the Campulosus-Sarracenia formation adjoins this community, fire will tend to open up the Ilex-Myrica complex to the savannah plants so that, provided the soil conditions are right, the Ilex-Myrica formation will be succeeded by the herbaceous savannah community.

6. **Campulosus-Sarracenia** *(Campulosus aromaticus, Sarracenia flava).* Savannah Land.

Distribution. Locally in that part of the lower or eastern half of the Coastal Plain lying south of the Hatteras axis. This formation occurs only on the practically level Chowan and Pamlico Terraces formed in Pleistocene time.

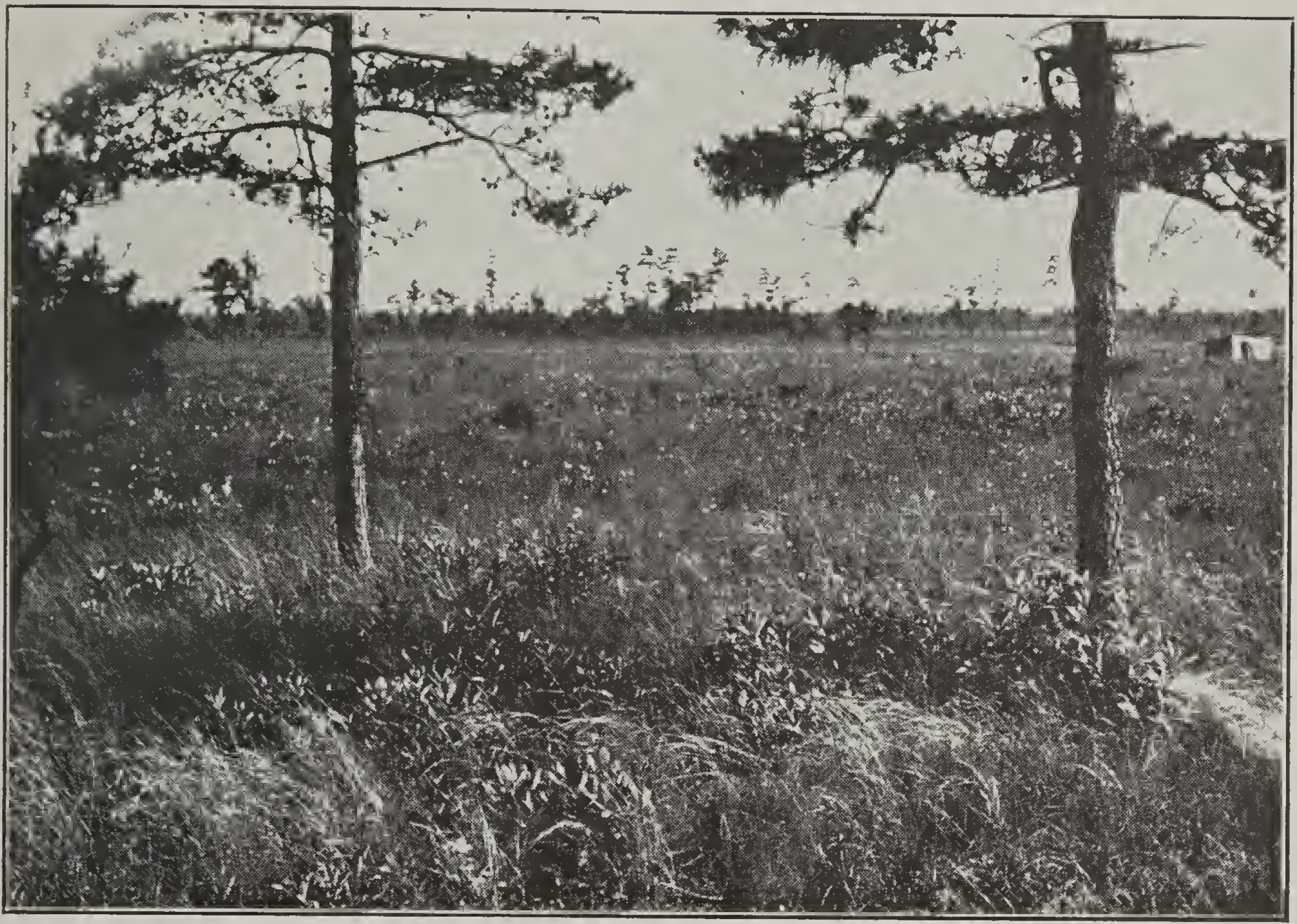

FIG. 3. Campulosis-Sarracenia Formation (Savannah)

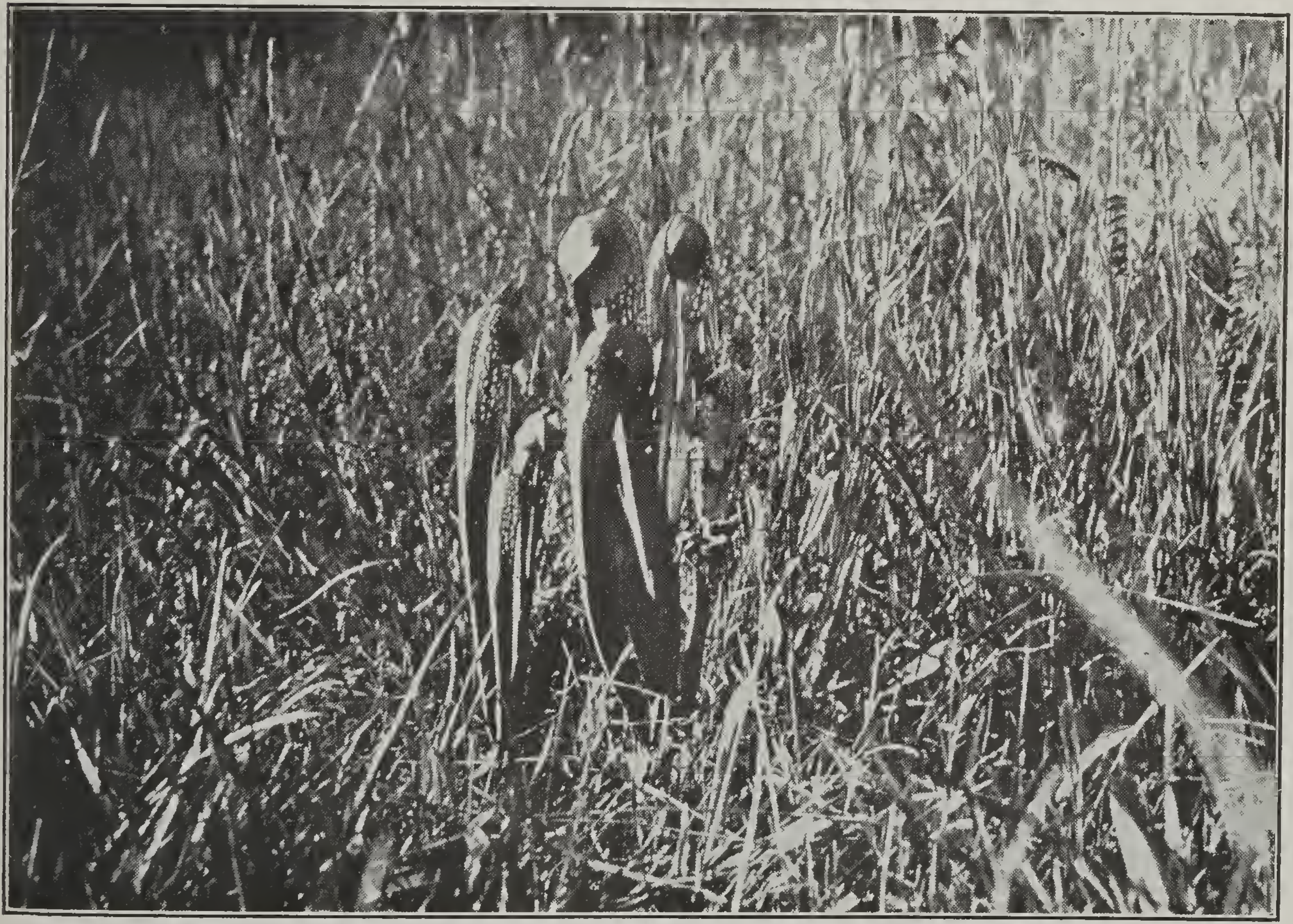

FIG. 4. Sarracenia minor, an element of the Savannah formation

Habitat. Level land with dark humus-filled, fine sandy soil underlain with a stiff non-draining subsoil. Free surface water, a few inches deep, is commonly present though in periods of extended drought, this may disappear. The soil under the latter condition may become baked into a hard caked layer, through the fact that the non-draining subsoil prevents the ascent of the water lying beneath it. It is thus seen that from a soil water standpoint, this is a habitat that undergoes marked changes.

Physiogonomy. All of the plants of this formation show definite xerophytic tendencies. They are almost uniformly herbaceous and of the types with buried stems. This latter character is correlated with fire survival. The commonest type, perhaps, of underground stem, is that of a primitive bulb in which the bases of the radicle leaves enter the ground to a depth of two to three inches, there joining the buried stem.

Associations. The very distinctive plants of this formation are so uniformly distributed in the uniform habitat that subcommunities of the rank of association are not clearly differentiated. A number of societies, however, may be mentioned:

Dichromena latifolia
Polygala lutea
Calopogon graminifolia
Aletris farinosa
Dionæa muscipula

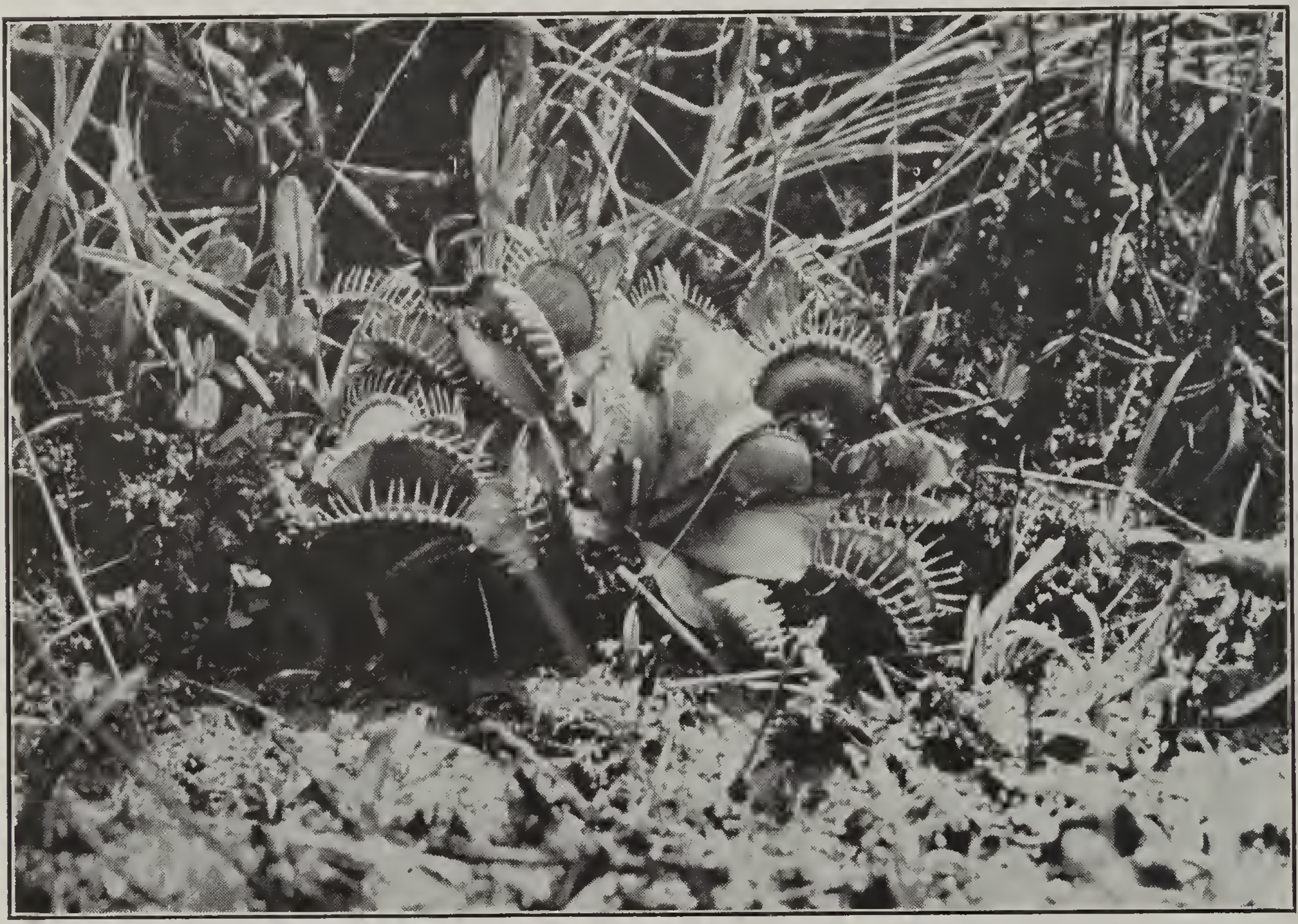

Fig. 5. Dionæa muscipula, a minor element in the Savannah formation

In the savannah vegetation, we have an example of bog xerophytism of a highly specialized type. It is very probable that this formation should be included in the same formation as that of the northern bogs. Until, however, sufficient work is done on both communities to solve definitely the relation of their xerophytism to the soil factors, it is deemed advisable to treat the savannah situation on a separate basis.

SUCCESSIONAL RELATIONS. There is little doubt that the savannahs in former times did not form such conspicuous entities as they now do. This was due to the fact that they formerly supported a large number of long leaf pine trees together with some loblolly and pocosin pine, which tended to hold in check the shade intolerant savannah species. The removal of these trees has made it possible for the distinctive herbaceous savannah vegetation to thoroughly master the areas it occupies, and if the soil conditions are right, with the aid of fire to also invade adjoining areas. In many places, the evidence is incontrovertible that the savannah complex is invading the Ilex-Myrica community.

As to the ultimate fate of these areas, nothing certain may be said. If they are undergoing an invasion of pines, it is extremely slow. Annual fires make practically impossible the establishment of pines, the only trees that can tolerate the soil conditions. So distinctive and permanent are some of the largest of these communties that they may be classed as subclimaxes. Or again, since there is no evidence of invasion, they might be regarded as edaphic climaxes. Much more study of the savannahs, however, must be made before any definite conclusions regarding their successional rôles may be reached.

7. **Nyssa-Taxodium** *(Nyssa aquatica, Taxodium distichum).* Swamp Forest.

DISTRIBUTION. Locally in the Coastal Plain, especially the eastern half.

HABITAT. Borders of lakes, ponds, and the smaller rivers. Soil high in humus, saturated, sand content variable.

PHYSIOGNOMY. Slight xerophytism is evident in the trees of this formation; especially prominent is the character of the enlarged or buttressed base, a feature associated with the soft yielding substratum in which these trees commonly grow. It must be acknowledged that the elements of this formation do not differ strikingly from their upland relatives, yet occupying the habitat they do, they naturally demand a place in a group of their own.

Associations. In addition to the two communities of the dominants, the following should be mentioned:

Chamæcyparis thyoides	*Persea borbonia*
Nyssa biflora	*Arundinaria arundinaria*
Acer rubrum	*Tillandsia usnoides*

Successional Relations. Accompanying the slow aggradational changes, this formation gradually gives way to the Quercus-Pinus association of the mesophytic forest. Extensive areas of this formation occur which are maintained for long periods of time so that this community may be regarded as an edaphic sub-climax formation.

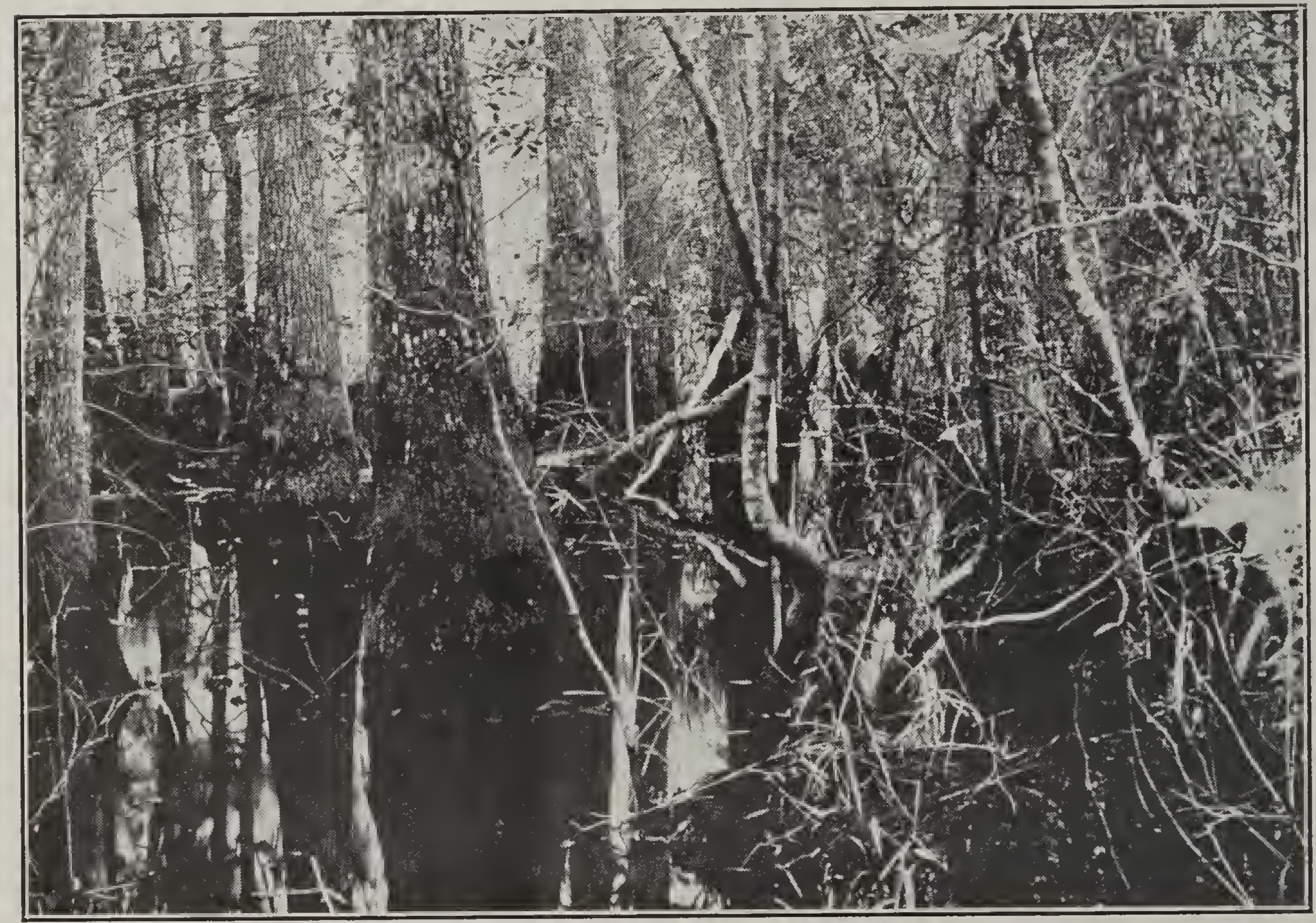

Fig. 6. Nyssa Association

8. **Quercus-Aristida** *(Quercus catesbæi, Aristida stricta).* Sand Ridge community.

Distribution. Locally in Coastal Plain south of the Hatteras axis.

Habitat. Well drained, humus-darkened, coarse, sandy soil, the surface one-half inch of which through the washing action of the rain, is humus-free, forming a glistening white layer. The surface of the sand ridges is thus as white as snow and the intense reflection of the midsummer heat and light constitutes the critical factor in the determination of the vegetation occupying the area.

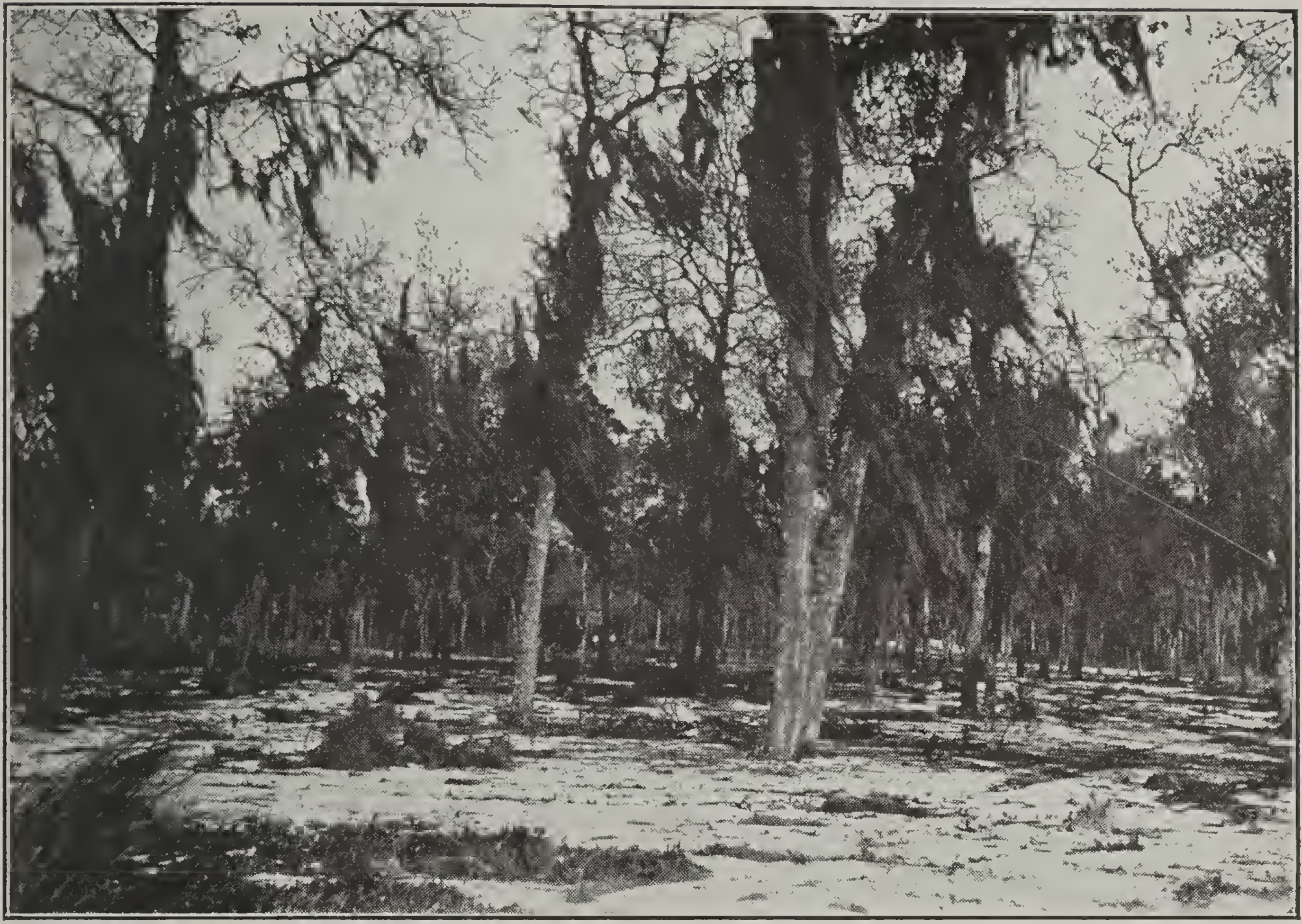

FIG. 7. Tillandsia in trees of Quercus Catesbæi on lee side of White Lake, N. C.

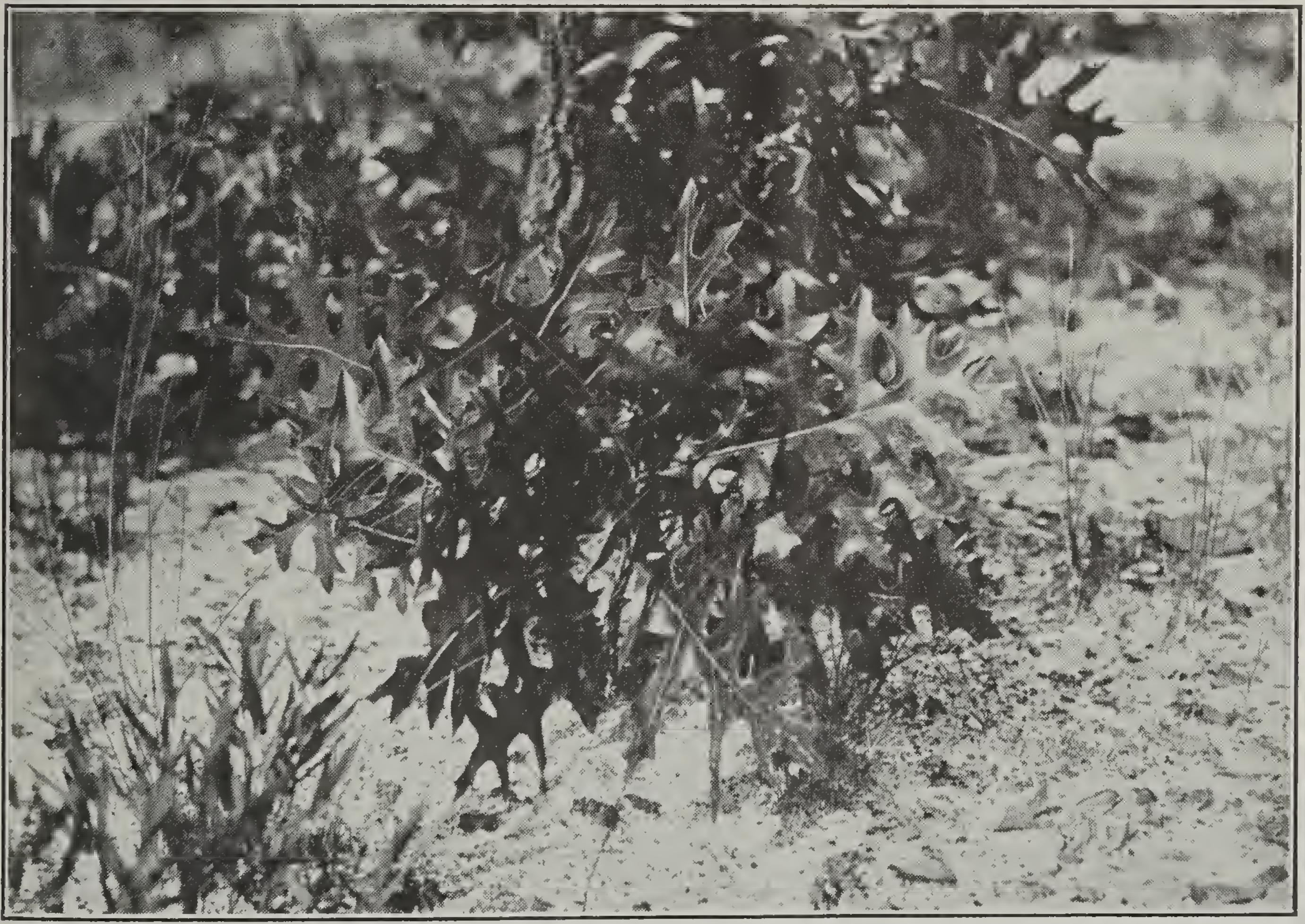

FIG. 8. Juvenile specimen of Quercus catesbæi showing vertical orientation of leaves

PHYSIOGNOMY. Definite xerophytism especially as this condition characterizes the juvenile stages. The young trees of the Turkey Oak (Q. catesbaei) and to a marked degree the old, as well, orient their leaves in a vertical position to escape the intense heat of the burning sands. The thin, erect tightly twisted, wire-like leaves of the wire-grass (Aristida stricta) are excellently adapted to meet the same extreme environment.

ASSOCIATIONS. The dominance of the two species mentioned is so complete that no other form is sufficiently prominent to constitute the basis of an association. Many societies of great ecological interest do occur, among the most striking of which are the following:

Stipulicida setacea
Lupinus diffusus
Cladonia sylvatica
Selaginella acanthonota

SUCCESSIONAL RELATIONS. Accompanying the development of the Aristida grass, which reduces the areas of shining sands, the formation tends to give way to the Quercus Marylandica association of the mesophytic forest. Following soon after or even accompanying this latter, Pinus palustris appears, which tree is destined to constitute the climax plant on these sandy areas.

9. **Syntherisma-Erigeron-Andropogon** *(Syntherisma sanguinale, Erigeron spp., Andropogon virginicus).* Old field, meadow, and roadside vegetation.

DISTRIBUTION. Locally throughout the State.

HABITAT. Upland sunny areas formerly cultivated or the original vegetation otherwise destroyed. Soils varying widely in water, humus, and mineral content.

PHYSIOGNOMY. Narrow leaved herbs (sun adapted), those on dryer soils showing xerophytic characters; on moist soils, hydrophytic ones.

ASSOCIATIONS. A large number of associations are found in this formation of which the following are especially prominent in addition to the three dominants chosen:

Ambrosia artemisiifolia
Eupatorium capillifolium
Aster tradescanti
Helenium tenuifolium
Daucus carota
Chrysanthemum leucanthemum
Paspalum spp.

While this formation is not so definite as most of the other developmental formations, due to the fact that many of its elements are involved in open tree associations, it has nevertheless in actual field experience proved to be a valuable concept.

SUCCESSIONAL RELATIONS. In the Piedmont and eastern sections there is no more striking succession than that among the dominants mentioned, in the name of the formation. In old fields everywhere may be seen the evidence of progress from the primary Syntherisma stage through the Erigeron, Eupatorium, Aster associations to the more permanent association of the one dominant Andropogon. This latter in time is almost always succeeded by a Pinus association.

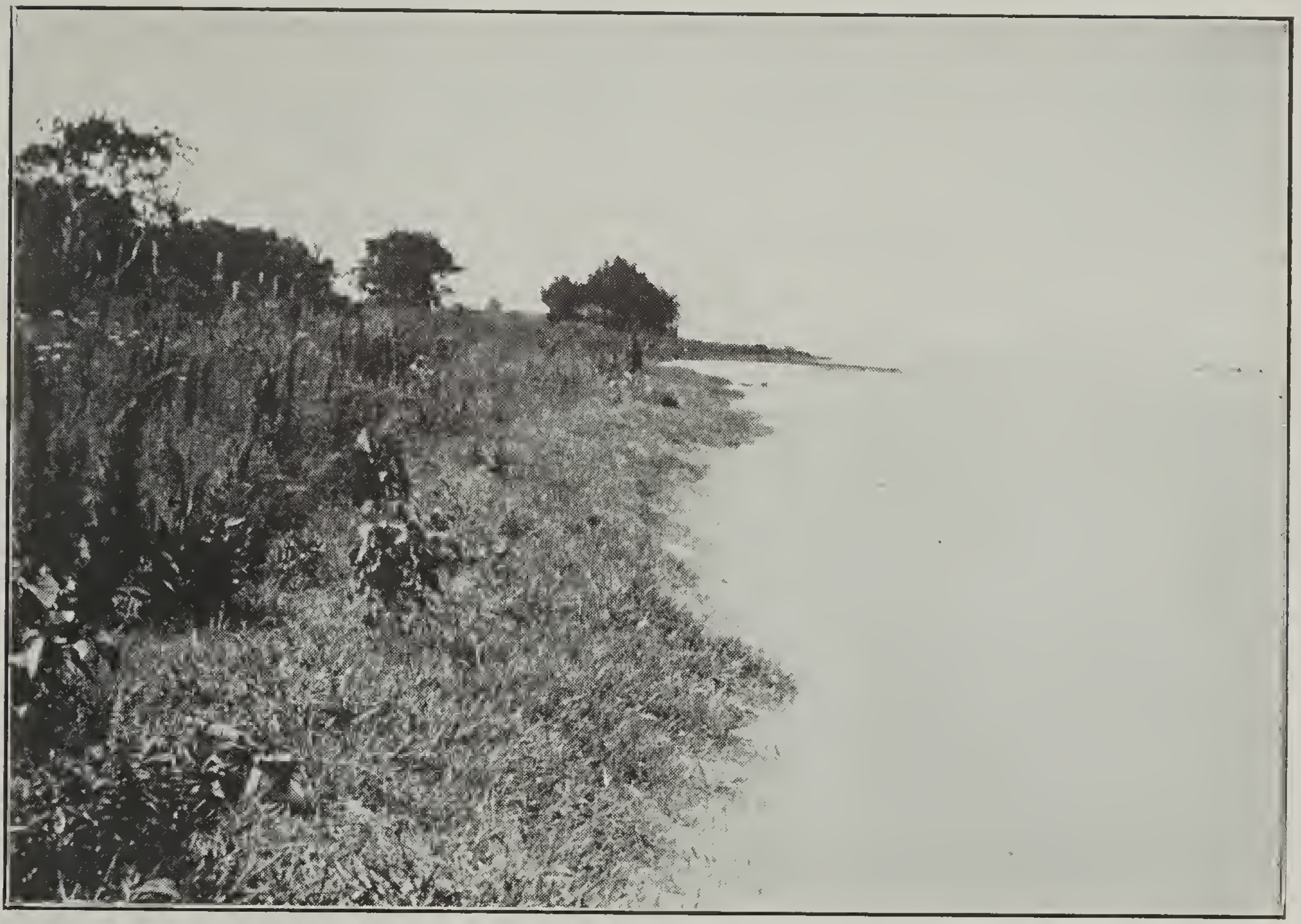

FIG. 9. Paspalum Association along Sound shore Currituck Peninsula

Quercus-Acer-Pinus Formation *(Q. alba, Acer rubrum-Pinus palustris).* Upland mesophytic forest.

DISTRIBUTION. General throughout the State below 6,000 feet.

HABITAT. Upland mesophytic conditions.

PHYSIOGNOMY. Dominants are broad-leaved woody plants and mesophytic conifers. Associated with the preceding are numerous shade tolerant herbs.

ASSOCIATIONS. Some of the principal associations are:

- *Rhododendron catawbiense-Alnus alnobetula.* Mountain "balds." Developmental or subclimax.
- *Quercus rubra.* Chiefly between 4,000 and 5,500 ft. Developmental or sub-climax.
- *Castanea dentata-Quercus velutina.* Chiefly between 2,500 and 5,000 ft. Developmental or subclimax.

Quercus alba-Quercus falcata. Fertile soils. Chiefly below 3,000 feet. Developmental, rarely subclimax.

Betula nigra-Platanus occidentalis. Chiefly on flood plains. Developmental.

Salix nigra-Alnus rugosa. Stream borders. Developmental.

Rubus sps. Clearings. Developmental.

Acer rubrum-Fagus grandifolia. Fertile soils below 5,500 ft. Climatic climax.

Quercus marylandica-Quercus stellata. Sterile soils. Developmental.

Quercus virginiana-Juniperus virginiana. Old coastal dunes. Developmental, subclimax.

Liquidambar styraciflua-Acer rubrum. Developmental.

Pinus palustris-Pinus tæda. Fertile soils. Developmental. Sterile soils. Subclimax. Edaphic climax.

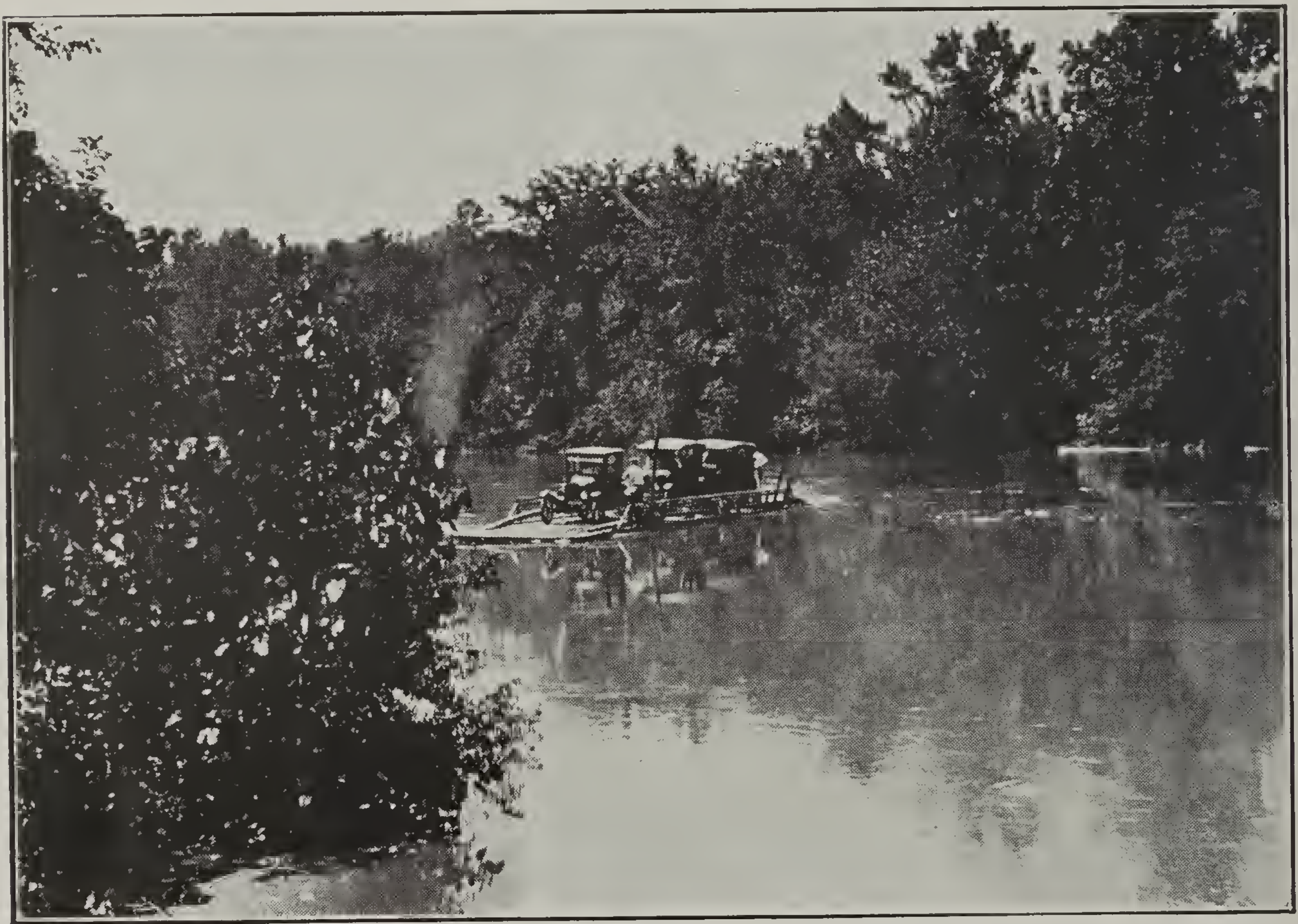

Fig. 10. Quercus association on fertile soil of slopes of the Cape Fear River in the heart of the Coastal Plain

It will be noted in the above treatment that the Acer-Fagus and Pinus climaxes have been included as associations in the mesophytic forest formation. To the mind of some ecologists such a "lumping" will seem quite unwarranted. To the writer, however, it seems inevitable if one is to be guided by the dual definition of major distinctive vegetation associated with a major distinctive habitat.

To place the *Pinus spp.* communities in a separate formation (fundamental ecological group) merely because their limits of tolerance of sterile soils are sufficiently broader than the broad-leaved forest elements to make possible their relative isolation, is not in the mind of the writer, adhering to the definition of the basic ecological community. Under the author's arrangement, Pinus palustris, tæda and others when found playing a successional role as they invariably do on fertile soils, constitute a developmental association. When these same species are found playing a climactic one on the humus-deficient upland soils of the Coastal Plain, they there constitute edaphic climax associations. It is thus far more logical to distinguish these two communities in such a manner than to attempt placing them in two formations when the habitat differences are minor ones and the physiogonomic differences are entirely lacking since the same species are involved.

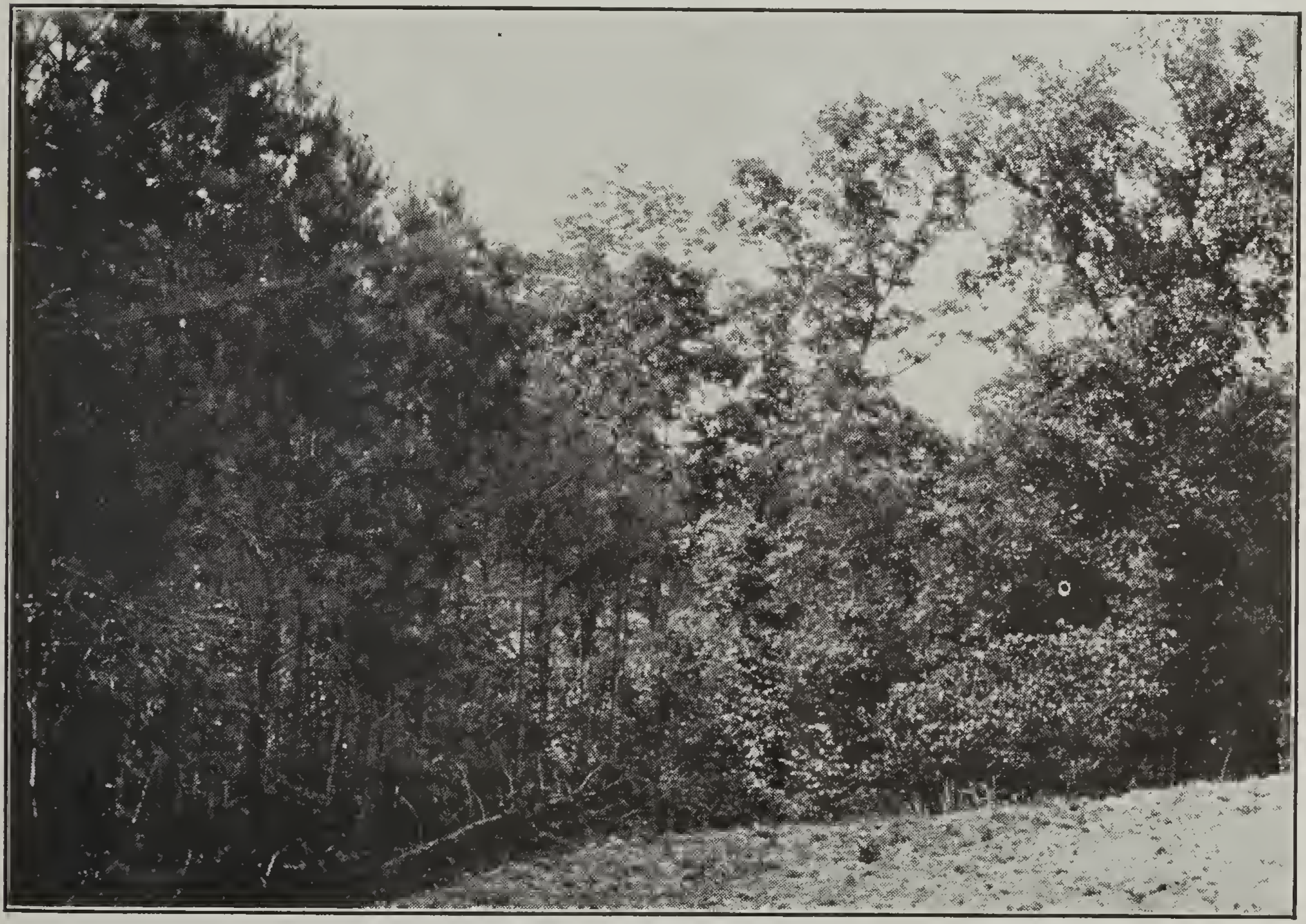

Fig. 11. Contact between developmental Pinus Association and Quercus-Carya Association

A further line of argument tending to indicate the validity of the writer's position is that related to the large number of mesophytic broad-leaved elements which actually occur associated as subdominants in the Coastal pine forests. The Quercus marylandica-Q. stellata association is commonly involved in them. In old forests such distinctly mesophytic elements as Q. falcata and Cynoxylon florida are frequently seen.

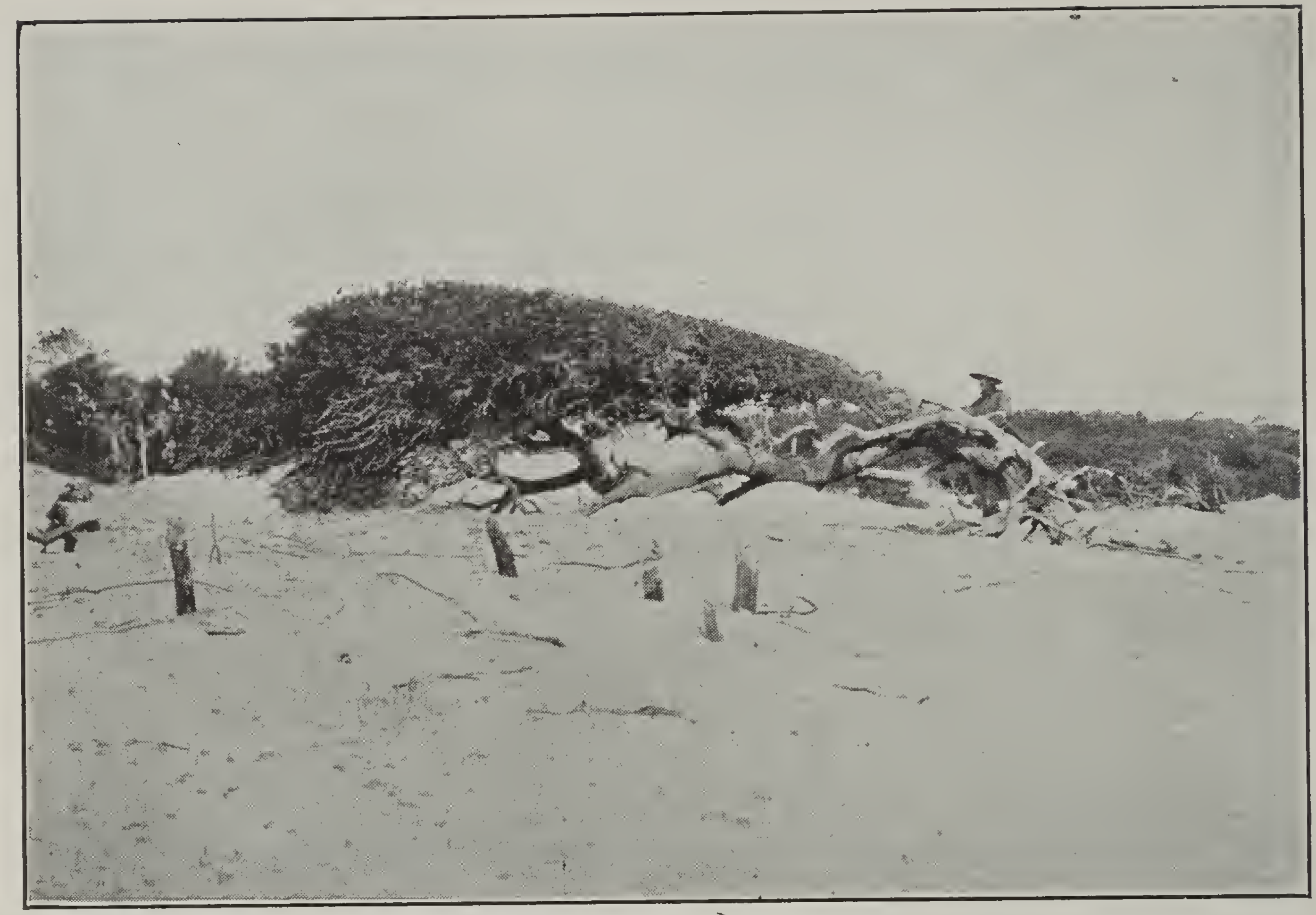

Fig. 13. Specimen of Quercus Virginiana on northeast facing coast near Nags Head

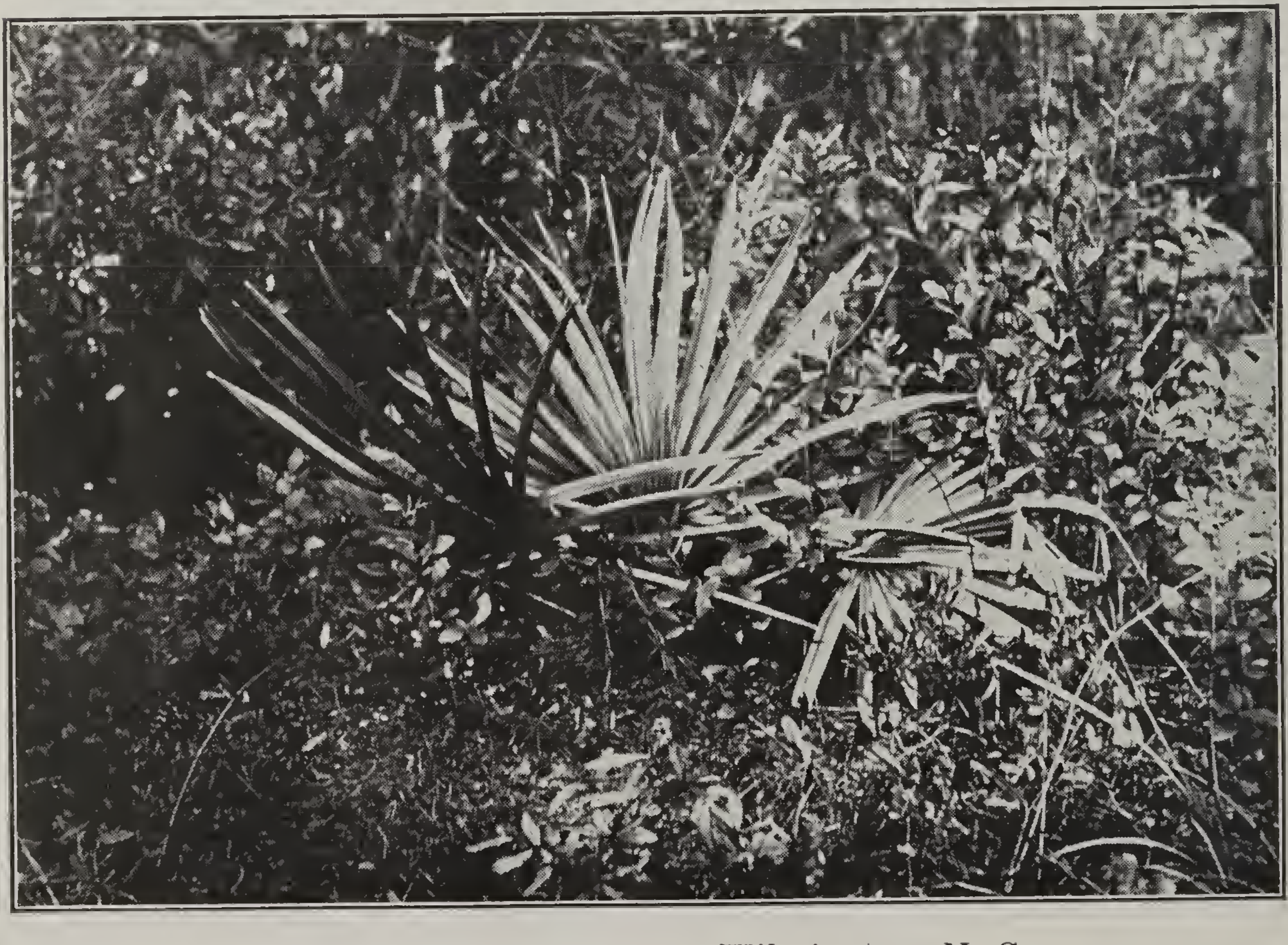

Fig. 12. Sabal Sabal near Wilmington, N. C.

Living on the edge of the Coastal Plain, the writer has had much opportunity to study the reactions and successional relations of the pines, and it now seems very clear to him that either a non-ecological definition for formation must be given, or if a truly ecological statement is made, the southeastern pine communities must be included in the mesophytic forest. This latter be it noted, is a water relation while the Coastal pine climax stands out for a soil sterility reason. There is thus no logical argument against placing the pine communities developmental and climactic under the great upland formation of woody mesophytes.

SUCCESSIONAL RELATIONS. Only the interformational relations will be briefly touched upon here.

Various associations of this formation commonly succeed the divisions of the Syntherisma-Erigeron-Andropogon Formation. The Quercus Marylandica-Q. stellata association is the first to enter the Quercus catesbiae-Aristida stricta Formation. A Quercus or Pinus association may follow the Nyssa-Taxodium formation upon filling in or draining. The Ilex-Myrica formation, it is believed, may slowly go over to the Pinus community, but sufficient data has not yet been gathered to indicate satisfactorily the major successional phenomena in relation to the bayland vegetation.

The shrub successions following the shallow water herbs is of course well known.

The maritime *Quercus virginiana-Juniperus virginiana* association is commonly observed to succeed the Uniola-Cakile formation on old dunes.

On fertile soils throughout the State except in the high mountain areas the developmental forest associations tend to go over into the Acer-Fagus climax association.

On the sterile soils of the Piedmont and Coastal Plain the Pinus association becomes isolated constituting subclimaxes in the Piedmont, while in the highly sterile soils of the Coastal Plain the Pinus association becomes a permanent edaphic climax.

Abies-Picea Formation *(Abies Fraseri-Picea australis).* Boreal Forest.

DISTRIBUTION. Above 5,500 ft. in the western part of the State.

HABITAT. Cold, moist mountain summits.

PHYSIOGNOMY. Chiefly evergreen trees with relatively heavy leaves. Certain minor deciduous elements which are adapted to the cold climate are also to be included.

ASSOCIATIONS. The two dominants are distributed in definite zones with the Abies highest. On the floor of the fir forest should be mentioned the Hylocomium-Oxalis layer society. As successional associations the Sambucus racemosus association and the Prunus pennsylvanica association are prominent.

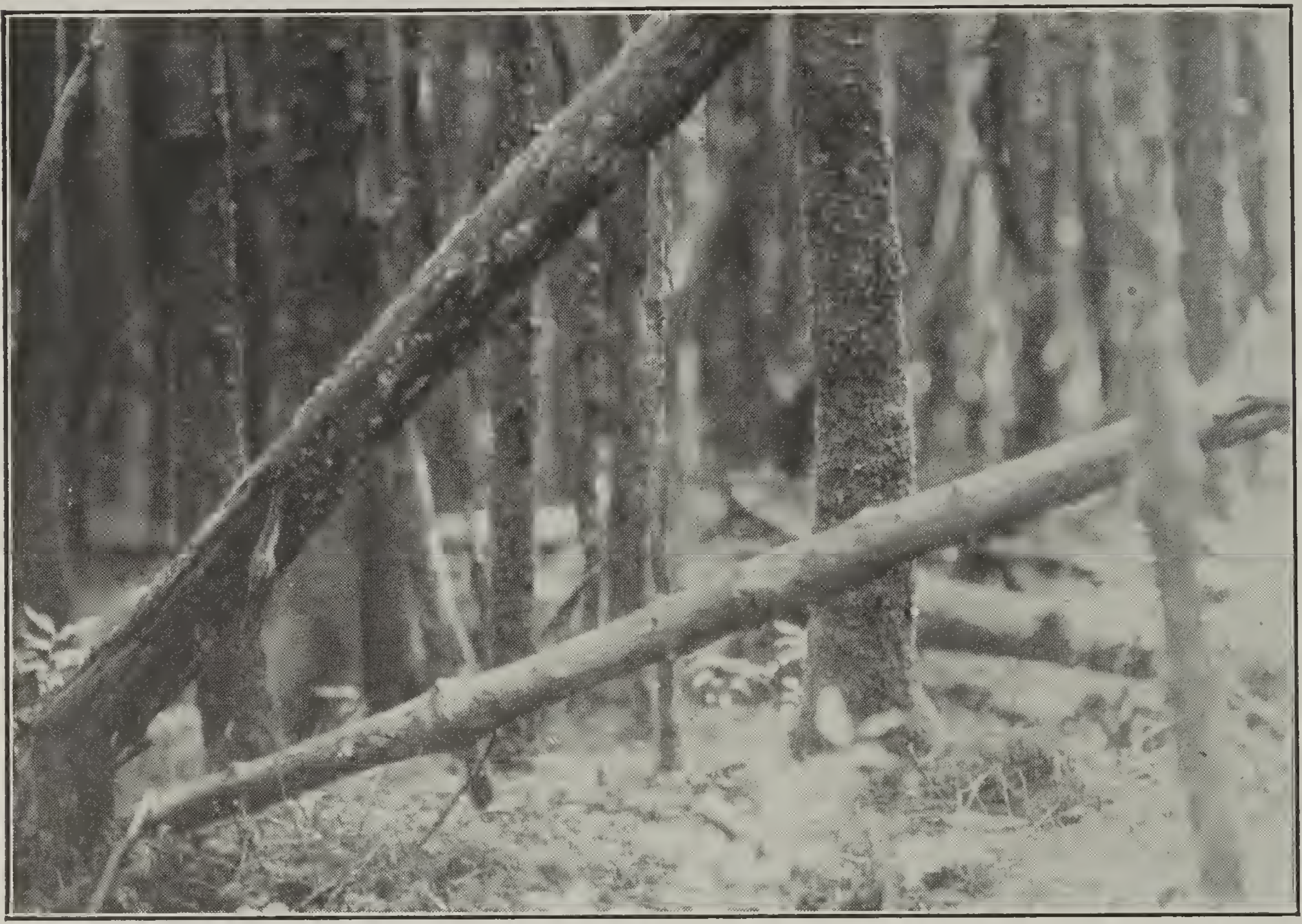

FIG. 14. Pure stand of Abies Fraseri on summit of Clingman's Dome in the Smoky Mountains

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JULY, 1925 TECHNICAL BULLETIN 26

STUDIES ON TREATMENT OF COTTON SEED

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BULLETINS OF THE STATION WILL BE SENT FREE TO CITIZENS OF THE STATE

SUMMARY

1. Anthracnose is one of the most destructive diseases of cotton in the United States. This disease causes losses amounting to many thousands of dollars annually in North Carolina alone. It is caused by a fungus which, under present cultural practices, is known to be spread chiefly in the form of spores and mycelium lodged upon the surface and within the cotton seed.

2. The methods commonly recommended for the control of seed-borne anthracnose of cotton are selection of seed from healthy plants, seed sterilization with chemical disinfectants, corrosives, and hot water and use of old seed for planting. Each of these methods is encumbered by certain defects which make desirable the development of a more certain and more effective method of control.

3. Other investigators have with more or less success attempted to control seed-borne diseases of other plants by the use of dry heat.

4. This study on the control of cotton anthracnose began with the use of seed of the 1919 crop and a new supply of infected seed was obtained each season. The seeds were heated in quantities of 50 or 100 in an electric oven provided with a constant temperature regulator. They were germinated individually on moist blotting paper in test tubes and each diseased seedling was examined microscopically to determine the nature of the fungus attacking it.

5. Certain preliminary experiments dealing with germination of cotton seeds and growth of the anthracnose fungus show that temperatures of 25 and 30° C. are not only very favorable for germination of cotton seed and the subsequent growth of the seedlings but also closely approximate the optimum for growth in culture of the fungus causing cotton anthracnose and for the development of the disease on seedlings. A temperature of 35° C. permits good germination of cotton seed but inhibits the growth of the fungus which causes anthracnose and prevents the development of the disease. At 20° C. germination of cotton seed and growth of the seedlings are good, but growth of the anthracnose fungus and the development of the disease are slower than at higher temperatures.

6. The germinability of air dry cotton seed was completely destroyed in 15 minutes by dry heat at 90 and 100° C. and seriously impaired after one hour at 80° C. A temperature of 70° C. for 24 hours impaired the germination of seed containing an amount of moisture normal

to ordinary storage conditions, but did not reduce germination of seed whose water content had been reduced to 8.3 per cent by storage under laboratory conditions. Anthracnose was greatly reduced by heating seed at 70° C. for 24 hours but was not completely eliminated by treatment at this temperature for 48 hours.

7. A temperature of 60° C. applied to air dry cotton seed either directly or after a period of preliminary drying at a lower temperature did not kill all the anthracnose.

8. Cotton seed which had been predried at temperatures of 40 to 50° C. for 24 hours or more were subjected to a temperature of 70° C. for 72 hours without marked loss of viability, but this treatment did not free the seed from anthracnose.

9. When infected cotton seed were predried at 40 to 45° C. for 24 to 72 hours or at 50° C. for 12 to 24 hours and heated at 80° for 24 to 72 hours, complete elimination of anthracnose was obtained in every test except one (5 out of 6) in which the heating was continued to 72 hours, but was secured in none of the tests in which heating stopped at the end of 48 hours. Little or no reduction of germination occurred as a result of these treatments.

10. When predried cotton seed were heated at 90° C., all anthracnose was killed in 24 hours in 7 out of 8 tests, in less than 24 hours in 7 out of 13 tests, and in 48 or 72 hours in the 3 remaining tests. No significant reduction in germination resulted from the treatment at 90° C. when the seed had been predried at 50° C. or above for 24 hours.

11. When predried cotton seed were heated at 100° C., complete control of anthracnose was secured in all except one (7 out of 8) test, and in this one instance the seed were heated for only 2 hours. Heating at 100° greatly reduced the germinability of the seed which had been predried at 50° C. or lower for 24 hours. Much better protection was obtained by predrying the seed at 60° C. for 24 hours.

12. When predried seed were heated at 95° C., complete control of anthracnose was secured in all of 14 tests in which the seed were heated longer than 8 hours, and in 4 out of the 6 remaining tests in which the seed were heated for 8 hours or less. Predrying the seed at 50° C. for 36 hours or at 60° for 18 to 24 hours conditioned the seed to endure the temperature of 95° C. without reduction in percentage of germination.

13. A machine for treating cotton seed in bulk with dry heat is described and illustrated. This machine is dependable and partially automatic in operation and requires only occasional attention of the operator. The viability of the seed-borne elements of the anthracnose

fungus present in infected seed may be destroyed by use of this machine. In this machine, the effective treatment for control of the disease without serious diminution of germinability consists of 20 to 24 hours of desiccation at 60-65° C. followed by 12 hours of heating at 95-100° C.

14. The moisture content of cotton seeds is a factor which strongly modifies their resistance to heat and which may be used as a criterion of their ability to withstand the temperatures that are effective in the control of anthracnose. Without exception, when the moisture content after the preliminary drying was as great as 3.9 per cent of the oven dry weight, the viability of the seeds was seriously impaired by heating at 95° for 12 hours. When the moisture content after the preliminary drying was not greater than 3.62 per cent of the dry weight of the seeds, no serious loss of viability resulted from the treatment at 95°.

15. When the water content after drying was not greater than 3.19 per cent of their dry weight, the seeds heated at 95° for 12 hours germinated more promptly than the untreated seeds used for a check. This accelerated germination is not due to any wholesome effect of the treatment on the embryo of the seed, but is to be attributed to changes in the seed coat which facilitate access of water to the embryo. Naked embryos from treated seed germinate somewhat less promptly than treated embryos, but when seeds are germinated with coats intact, this effect is completely masked and amended by the favorable changes in the seed coat.

16. The viability of the seed-borne elements of the anthracnose fungus was prolonged by storing infected seed over such desiccating chemicals as concentrated H_2SO_4 and CaO. This result shows that the death of the anthracnose fungus when infected seeds are treated with dry heat is not due to the desiccation accompanying the treatment but to a more direct effect of the heat on the fungus protoplasm. The necessary preliminary desiccation serves only to prepare the seeds to endure the high temperature used in the final stage of the treatment.

17. The thorough desiccation which occurs when cotton seeds are stored for long periods over concentrated H_2SO_4 and dry CaO, induces a condition of more or less complete secondary dormancy in many of the seeds. This condition is slowly reversible and is characterized by a very hard and dry condition of the seed coat which prevents absorption of water. The embryos of such seed germinate promptly upon removal of the seed coat.

18. The storage of infected cotton seed in hydrogen and carbon dioxide does not free the seeds from anthracnose.

STUDIES ON TREATMENT OF COTTON SEED

By Samuel G. Lehman

PREFATORY STATEMENT

This investigation has, from its inception in 1920, been concerned primarily with the control of cotton anthracnose, although studies have been made of a number of other seed-borne diseases. Consideration has been given mainly to treatment with dry heat and, as might be anticipated, high temperatures have been found to influence not only the viability of the parasite but also the germination of the cotton seed themselves. The study has included the influence and interrelationship of the several factors, degree of heat, duration of the period of heating and moisture content of the seed, on germination, enzymic activity and respiration of the seed and on the viability of the fungus. Since the work on those portions dealing with the influence of dry heat on enzymic and respiratory activity is not yet completed, it is planned to report on them in the near future as separate series of these "Studies on Treatment of Cotton Seed" and to include in the present papers the completed phases of this work. These data naturally group themselves under two general headings, (1) The control of cotton anthracnose by the use of dry heat and (2) Germination of cotton seed and viability of anthracnose as influenced by desiccation and by storage in inert gases.

ECONOMIC IMPORTANCE OF COTTON ANTHRACNOSE

Anthracnose of cotton is one of the most destructive of all the diseases common to this important plant in the United States. As the result of the presence of this disease, cotton growers, breeders and seedsmen suffer loses amounting to thousands of dollars annually. Destruction of 30 to 50 per cent of the bolls in some fields is not an infrequent occurrence and cases are known where the losses amounted to 80 or 90 per cent of the crop. A fair idea of the amount of loss caused by this disease may be gained from a consideration of estimates published by the Plant Disease Survey.[26] These figures show that in 1923 all cotton diseases combined caused a reduction in yield of 2,439,000 bales in the United States. Nearly one-tenth of this loss, or a reduction of 229,000 bales is attributed to anthracnose alone. In the same year, the loss to North Carolina cotton producers caused by anthracnose amounted to 36,000 bales. These estimates do not include the concomitant reduction in yield of seed nor the losses arising from the destruction of the stand during the seedling stage. As a result of the use of diseased seed, it is often necessary to replant entire fields and in consequence to lose valuable time and labor from other urgent springtime operations.

Cotton anthracnose is caused by the fungus *Glomerella gossypii.* This fungus attacks the cotton plant in all stages of its development. If infected seed have been planted the seedlings will be attacked and diseased areas will appear on cotyledons and stems. If cool, moist weather prevails most of the diseased seedlings will perish. More favorable weather conditions make it possible for many of the diseased seedlings to survive the attack of the fungus and to grow to mature plants. Such plants are, however, not free from the disease, but, on the contrary, bear many diseased bolls and serve as centers from which neighboring plants may become infected.

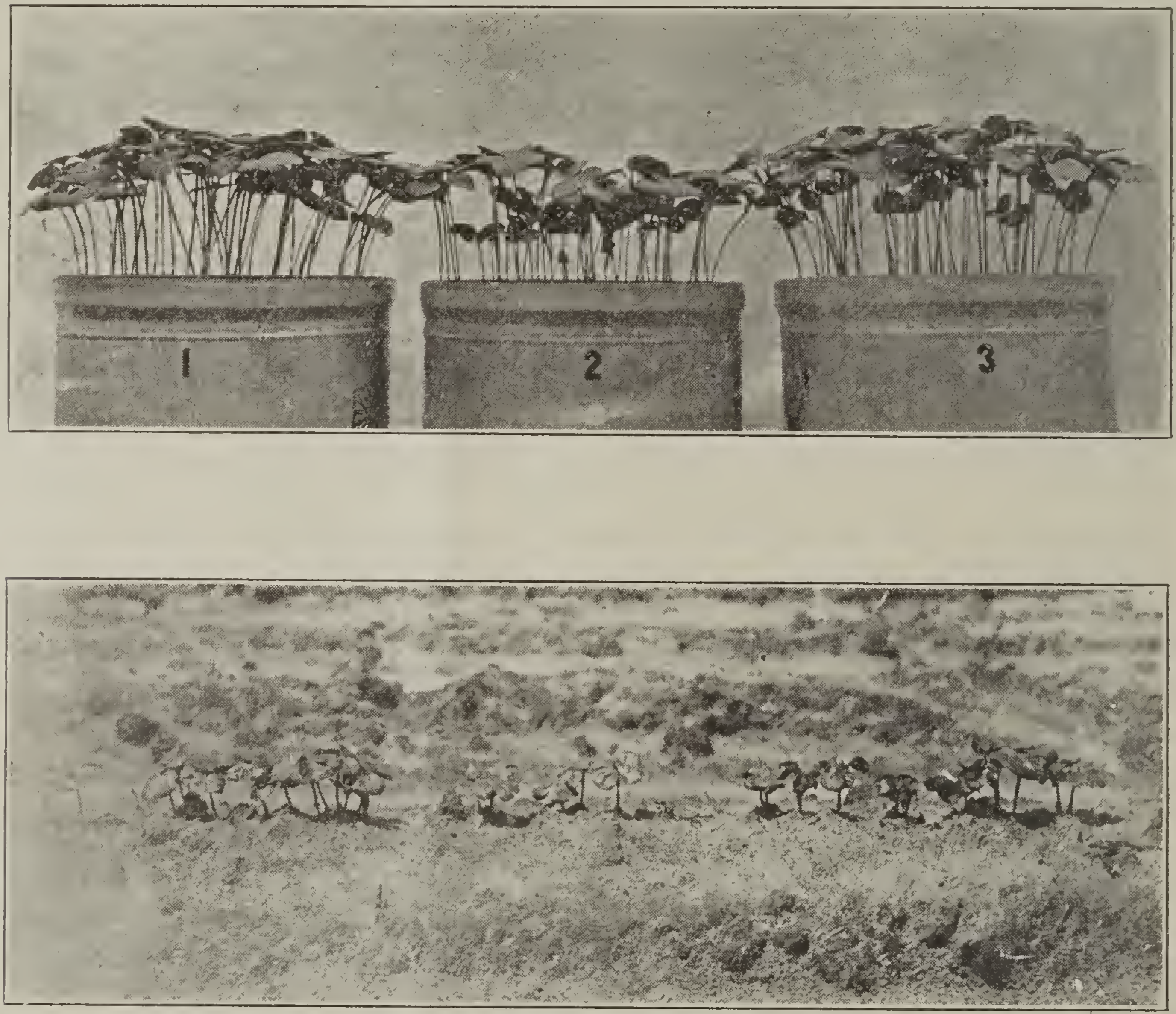

Plate 3 Top. Cotton seeds treated in the machine described in this bulletin and planted in jars in the greenhouse. 50 seeds were planted in each jar and the picture was taken 11 days later. The seed planted in jar 1, had been dried at an average temperature of 51° C. for 24 hours then heated at 90° C. for 22 hours. The seed planted in jar 3 had been dried at 40-45° C. for 24 hours, and subsequently heated at 80° C. for 72 hours. The seedlings in pot 3 came from a comparable lot of untreated seed. Germination was as follows: jar 1, 96 per cent; jar 2, 98 per cent; jar 3, 98 per cent. The treated seed germinated as well and produced somewhat more vigorous seedlings than untreated seed.

Bottom. A close-up view of some of the plants shown in Plate 4.

While cotton is growing from the seedling to the fruiting stage the fungus lives and sporulates on the cotyledons, stems, leaves and leaf scars. It has recently been reported[16] that anthracnose grows in the nectar glands of leaves of infected plants. This may serve as another source of infection for bolls which develop later. The greatest and most obvious loss arises from damage to the bolls. The fungus grows within the tissues of the bolls and produces more or less discoloration or even complete decay of both fiber and seeds. Individual bolls may fail entirely to open or if less severely attacked only a part of the locks may open. In such cases, the lint is frequently so badly discolored or so firmly matted together as to be worthless. Even when infection is so slight as to produce no apparent discoloration, the microscope will reveal the presence of the fungus filaments among the fibres and within the tissues of the seed.

NATURE OF THE SEED-BORNE INFECTION

By the planting of infected seed, it is easily demonstrable that the fungus which causes cotton anthracnose is carried from season to season on the seed. This fact may be established by germinating on sterile blotters seed which came from diseased bolls. Some or all of the seedlings will develop anthracnose lesions on cotyledons or hypocotyl, whereas seed from uninfected bolls will produce healthy seedlings only. A large portion of this inoculum is carried in the form of spores and mycelium in the fuzz on the surface of the seed. If diseased seed which have been delinted with sulphuric acid are germinated it is found that the percentage of diseased seedlings is very much less than that obtained by germinating untreated seed from the same source. However, the most severe treatment with surface disinfectants will not kill all the seed borne elements of this disease. This situation indicates that the fungus causing anthracnose must be carried in part within the seed. Barre[3] has described and clearly illustrated this condition by drawings made from sections of diseased seeds which show that the mycelium penetrates the seed coat and invades the tissues of the embryo often sporulating on its surface. Here it remains in a dormant but viable condition throughout the winter and starts into active growth when the seeds germinate in the following spring. Any seed treatment, therefore, which is directed toward the control of cotton anthracnose must reckon with the the internal nature of the infection in order to attain any satisfactory measure of success.

METHODS COMMONLY RECOMMENDED FOR CONTROL OF ANTHRACNOSE

A. *Seed selection.* Field selection of seed is a practice which cannot be too highly recommended as a matter of general farm procedure. It is advisable from an agronomic as well as a pathological point of view. Very encouraging results have been obtained in the control of cotton anthracnose by taking seed from plants which bear no diseased bolls and which are not adjacent to other diseased plants. By selecting seed from healthy plants in a field where 20 per cent of the bolls were infected Barre[3] obtained a crop free from anthracnose, whereas the crop from unselected seed from the same field was abundantly diseased. However, when the average person, whose eye is not trained to recognize infections of an incipient or minor nature, makes the selections, a small amount of diseased seed is liable to be included unwittingly. Moreover, in cotton breeding operations small plots representing strain or type selections may become diseased as the result of wind or insect borne spores from neighhoring fields. If, in such a case, one is forced to select seed from only a part of the plants because of the presence of disease on the others, the quantity of seed available for the multiplication plots is greatly reduced. Seedsmen and breeders who have had this experience appreciate the desirability of a dependable method of treatment for killing all seed-borne infection without reducing the capacity of the treated seed for germination. Moreover, this would make possible the introduction with safety of seed of desirable lineage whose disease record is unknown.

B. *Surface disinfection.* Treatment of diseased cotton seed with such surface disinfectants as solutions of copper sulphate, mercuric chloride and formaldehyde is difficult because of the fuzz on the surface of the seed. These solutions do not readily enter the interstices of the fuzz; hence a large portion of the surface borne infection escapes their action[4, 10, 33]. Moreover, such disinfectants cannot reach the elements of the fungus carried within the embryonic tissues without seriously damaging the embryo itself.

Delinting with sulphuric acid has also been used as a measure of control of cotton anthracnose. This solution dissolves not only the lint, but also any spores and mycelium that may be carried in it. Such treatment greatly diminishes the load of infective material carried by the seed and decreases the incidence of the disease in the field. However, it does not render infected seed completely free from disease since delinting does not reach mycelium and spores carried within the seed. This fact is clearly demonstrated by tests made by the writer in which highly diseased seeds were delinted with sulphuric acid and compared with nondelinted seeds in order to determine the relative number of seeds in which the fungus had penetrated beyond the reach of this powerful disinfectant. Each test comprised 50 treated and 50 untreated seeds selected to exclude any which appared to be nonviable. The seeds were kept in concentrated sulphuric acid for periods of 10, 20 and 30 minutes. During this time they were repeatedly stirred to insure good contact with this acid. At the end of the periods indicated the seeds were washed in several changes of tap water to remove the acid and finally placed on sterile, moist blotting paper in test tubes to germinate. The result of these tests are presented in Table I.

TABLE I

Percentage of Anthracnose Which Developed on Seedlings From Corresponding Lots of Delinted and Undelinted Cotton Seed.

Lot No.	Minutes in Con. H_2SO_4	Days in Germinator	Per Cent of Seed Which Developed Diseased Seedlings		Per Cent of Germination		Year When Grown
			Treated	Check	Treated	Check	
1	20	8	6	66	64	98	1919
2	10	8	12	34	82	98	1919
3	10	8	10	34	70	98	1919
4	30	14	16	------------	92	------------	1922

In test 1, 6 per cent of the treated as compared with 66 per cent of the untreated seed produced diseased seedlings. Likewise, in tests 2 and 3, a marked reduction in number of diseased seedlings resulted from the treatment. However, in none of the four tests did delinting with sulphuric acid completely suppress the disease. With the 10 minute treatment a small amount of fuzz still persisted at the large end of the seed and this may have prevented the complete destruction of such infective material as may have lodged in it. However, with the 30 minute treatment, the seed coats were much corroded and the lint was so completely removed that it seemed impossible for any spores or surface borne material to survive. Crawford[17] supplemented the usual treatment with sulphuric acid by subjecting delinted, washed and dried seed to a second charring with sulphuric acid and a subsequent treatment with a 1-1000 solution of mercuric chloride. He reports that, when such seed were germinated on sterile blotting paper, cultures of *Glomerella gossypii Diplodia gossypii, Cephalothecium* sp., *Alternaria* sp. and *Fusarium vasinfectum* were obtained from them. These tests make obvious the conclusion that while seedling infection may be greatly diminished it cannot be completely eliminated by delinting.

C. *Hot water.* In 1910, Duggar and Cauthen[19] testing the efficiency of various seed treatments found that the amount of seed borne infection in the case of cotton anthracnose could be greatly lessened by immersing diseased seeds for 22 minutes in water of a temperature of 150° F. Of the bolls which grew on these treated plants, 2.4 per cent became diseased compared with 9.9 per cent on plants from untreated seed. The marked reduction in percentage of diseased bolls apparently resulting from this treatment aroused the hope that the complete elimination of seed-borne infection might be accomplished. In 1914, Barre and Aull[12] published a preliminary report of their work with the hot water treatment. They found that seeds could be kept in water at 70° C. for 15 minutes without injury to germination and, when so treated, diseased seed germinated free from disease, while disease appeared on 22 per cent of the seedlings from the untreated seed. However, in field tests of seed which had been treated at 75° C. for 15 minutes made during this and subsequent years,[5, 6, 12] a small amount of disease developed on the plants grown from treated seed. This amounted to much less than one per cent of the bolls on plants from treated seed, whereas 15 per cent of the bolls from untreated seed became diseased.

The method is encumbered by certain difficulties of operation. It appears that a temperature of less than 70° C. for fifteen minutes is too low to destroy the seed-borne infection, while a temperature of more than 75° C. for 15 minutes causes unfavorable reduction in germination of the seed treated[19, 12] Barre[6] has stated that some varieties of cotton are killed at 72° C. while others will tolerate 76° C. These close temperature limits for the killing of the seed and of the fungus make it difficult in practice to maintain the water at a proper temperature when seed are treated in quantity. This operation is troublesome since it involves a suitable place for drying the seed and considerable time and labor. Moreover, after cotton seed have been treated by the hot water method, they must be dried before they can be stored again. Thus, while the hot water treatment greatly diminishes the number of seeds carrying viable elements of the anthracnose organism and greatly reduces the incidence of the disease in the field, it does not appear that the method can be depended upon to completely eradicate seed-borne infection.

D. *Ageing of seed.* In 1910 Barre[8] planted seed of the 1908 crop on disease-free land and about 10 per cent of the bolls became diseased whereas plants from healthy seed remained free. Seed from the same lot was planted again in 1911, when less than one per cent of the bolls developed disease. Germination tests of this same seed revealed no diseased seedlings. This indicates that seeds carrying the anthracnose fungus in a viable condition are very few in number by the middle of the third year. In 1916[7] he stated that "Three-year-old seed kept in our office and laboratories continued to give disease-free plants when planted in the field," but that "some of the seed claimed by farmers to be three years old, and kept in sheds or barns did not give such satisfactory results. . . ."

Edgerton[20] made successive plantings of infected seed in the greenhouse and found that no diseased plantlets came from seeds which had attained the age of 13 months. Cultures made from seed gave results in harmony with those obtained from the greenhouse experiments. The writer's observations on this point show that seed of the 1919 crop, germinated in test tubes when 9 months old produced seedlings 58 per cent of which were diseased. On April 16, 1921, 18½ months from picking time, 4 per cent of the seeds still bore viable elements of the anthracnose fungus. During the nine months immediately following picking, these seed were stored in an old barn and were for the remaining 9½ months kept in a closed can in the laboratory. In another test, seed of the 1921 crop produced 20 per cent of infected seedlings when 17½ months old and 2 per cent when 21½ months old. In two tests made with seed which were heavily infected when harvested and which were older than 21½ months, no disease appeared on the seedlings. It should be stated, however, that these seed were stored in the laboratory and were not, therefore, subjected to the changes which obtain in gins and barns, places where seed are usually stored. In view of these observations, it seems probable that, for North Carolina conditions, diseased seed may be planted safely in the third season after picking.

Ageing is the only process yet described for completely freeing infected cotton seed from anthracnose. This method has certain objectionable features, however, the most obvious of which are:

1. Storage space must be provided for seed from three seasons' crops.
2. It quadruples the required storage period and proportionally increases the losses arising from fire, rains and rodents.
3. Breeders cannot by this method free their seed from infection without serious loss of time and very great inconvenience.

Consideration has been given in the above paragraphs to the various methods heretofore recommended as helpful in the control of seed-borne infection by anthracnose. None of these measures yield thoroughly satisfactory results under all conditions. There is evident need of a method of treatment which can be used at will to effectually destroy all seed-borne infection. It is the purpose of the present paper to present such a treatment for the elimination of cotton anthracnose from infected seed.

TREATMENT OF INFECTED SEEDS WITH DRY HEAT

Within the last two decades, investigators interested in the control of plant diseases have attempted to eliminate seed-borne infection by the application of heat, hoping to discover a differential tolerance of the seed and the invading fungus. In this work, both dry and moist heat have been employed with varying degrees of success. In 1908, Kuhle[22] stated that he was able to free barley of loose smut infection by the use of dry heat, but was only partially successful in eliminating wheat smut. By first drying wheat and barley seeds for short periods at successively higher low temperatures then finally subjecting them to dry heat at 90° C. to 110° for ten minutes, Störmer[31] decreased greatly the amount of loose smut on barley, but accomplished very little decrease of wheat smut. Temperatures of 80-100° applied for 45 minutes resulted in little or no reduction of smut in summer wheat and summer barley.[32] In 1911, Appel and Riehm[1] treated smut-infected seeds of wheat and barley by soaking them for periods of 2 to 8 hours then heating them for 10 minutes in water at 48-50° C. In the case of the longer periods of soaking, the per cent of smut was markedly reduced. In other trials, it was found that better control was obtained by heating in water for 10 minutes at 54-56° C. No disease developed when seed were permitted to imbibe about 17 per cent of water and were then heated for 20 minutes in an oven at 55-60° C. In 1916, Naumov,[25] attempting to develop a treatment for cereal seeds infected with fungi (*Gibberrella saubinetti*, *Fasarium roseum* and *F. subulatum*) believed to be the cause of the condition known in Russia as "intoxicating bread," heated rye at 66° C. and wheat, oats and barley at 60° C. for periods ranging from 24 hours to 3 days. He believed that by this method he was able to kill these seed-borne fungi without reducing the germinability of the seed. Atanasoff and Johnson,[2] however, in tests with scab of wheat and barley were unable to verify Naumov's experiments. However, when they subjected wheat, rye, oats and barley to treatment with dry heat at a temperature of 100° C. for 30 hours, they found that these seeds withstood the prolonged treatment remarkably well, the reduction of germination ranging between 10 and 40 per cent exclusive of a very few extreme cases. Moreover, seed-borne infections of the bacterial blight of oats and barley were killed and wheat scab and spot blotch of barley practically eliminated by this treatment. Also, the Helminthosporium blotch diseases and the loose smuts of oats and barley were greatly reduced. In 1922 Blodgett[14] heated Bliss Triumph potato tubers infected with mosaic in water at temperatures from 35 to 80° C. for varying times. He concluded that at the temperatures used the potatoes were killed in less time than was required to destroy the mosaic virus. In 1923 Schitikova[28] reported that dry heat at 60° C. to 66° C. for 24 to 48 hours and 85° for 30 minutes does not injure germination of wheat and is efficaceous against smut, but does not completely suppress the disease. In the same year, Doran[18] reported that heating diseased seed of the garden bean for two hours at 80° C. did not give as good control of bean anthracnose as other methods.

The marked success of the dry heat treatment in the control of cereal diseases led the writer to test the efficiency of this method for the elimination of cotton anthracnose from infected seed. The great reduction in anthracnose resulting from the hot water treatment noted above gave additional hope that the application of dry heat might prove effective in the treatment of diseased seed. Certain preliminary tests were accordingly begun in the spring of 1920. While this work was in progress Barre[9] and his colleagues, Lipscomb and Ludwig, at the South Carolina Experiment Station made brief reports on the progress of their studies of the physical factors influencing the vitality of the anthracnose fungus carried by diseased cotton seed. In 1919 Lipscomb[9, 10, 11] reduced the water content of infected seed from 9 to 2 per cent by desiccation in a vacuum and was led to believe that this treatment killed the fungus. He also reported that the "fungus is killed more readily by a combination of heating and drying than by desiccation alone." In 1920, Lipscomb and Ludwig[10] working with different lots of seed and different varieties of cotton were unable to confirm the findings of the previous year. In the following year, Ludwig[11] concludes as the result of various treatments such as drying in a electrical seed drier and hot air oven, sterilizing and storing in various situations, that none of these methods may be reled upon to reduce the infection to any marked extent.

In 1923 Lipscomb and Corley[23, 24] believing that cotton seed could not be successfully freed of anthracnose by heating in air because of harmful oxidation of the fats and proteins described a method developed by themselves whereby seed were subjected to high temperatures in the absence of oxygen. A special glass tube was devised within which the diseased seed and a quanity of CaCl were sealed in a vacuum of 1 mm. or less or in an atmosphere of nitrogen. After they had been allowed to dry, these seed were heated in this tube at a temperature of 100° C. for 26 hours. No injury resulted to the seed and the anthracnose was completely suppressed. Likewise, heating at 110° C. for 10 hours did little or no injury to the seed but destroyed all anthracnose. Exposures for periods of 1 and 3 hours at 110° C. and 1 hour at 120° C. greatly diminished but failed to completely eliminate the disease. This method although effective has the disadvantage of requiring that the seeds be heated in a vacuum or in some inert atmosphere.

MATERIALS AND METHODS

This work began in the summer of 1920 with the use of seed from the 1919 crop. Owing to the tendency of the seed-borne inoculum to lose its viability after one year, a new supply of infected seed was obtained each season. Cook was the variety used in 1923 and 1924; the names of the varieties used in other years are unknown. Fifty per cent or more of the seeds of all lots used were shown by tests to carry viable inoculum of the anthracnose fungus.

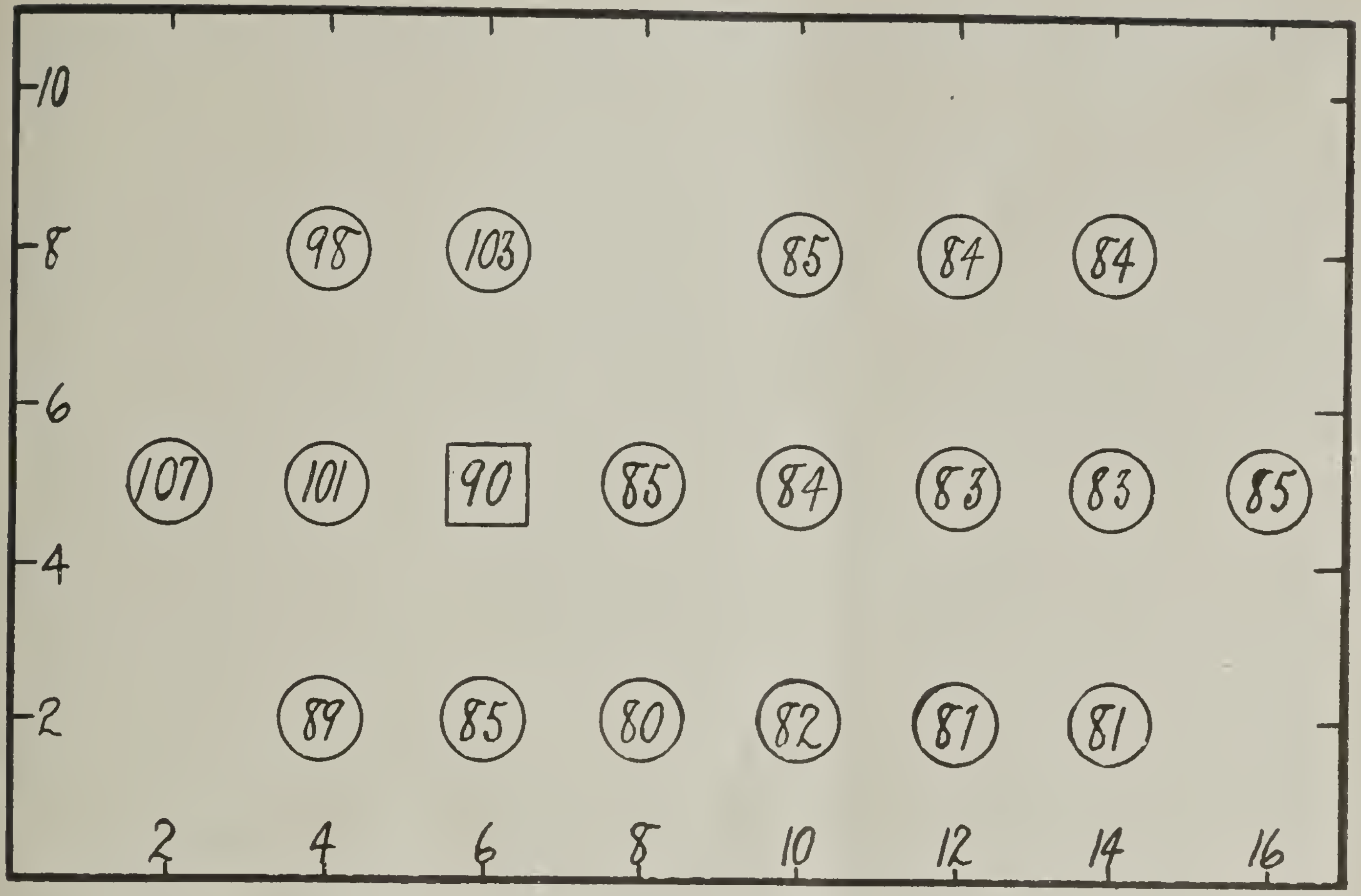
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FIG. 1. Map showing the variations in temperature which obtain between various positions on the shelf of the electrical oven. The numbers in the circles are the temperatures at the positions indicated by the circles when the oven thermometer at the place marked by the square stands at 90° C. The marginal numbers represent inches.

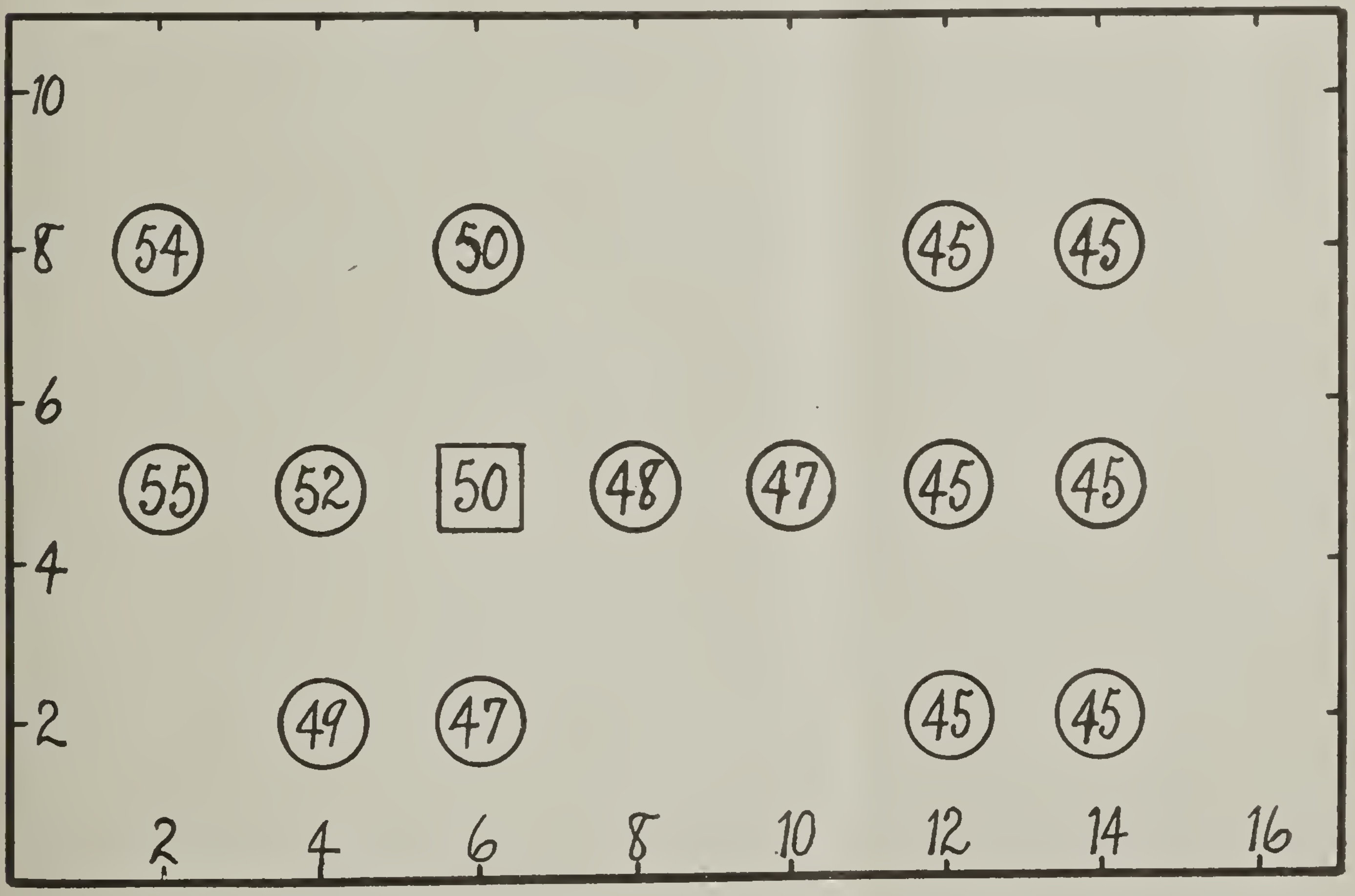
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FIG. 2. Map showing the variations in temperature which obtain between various positions on the shelf of the electrical oven. The numbers in the circles are the temperatures at the positions indicated by the circles when the oven thermometer at the position marked by the square stands at 50° C. The marginal numbers represent inches.

With the exception of a few preliminary tests made in a gas oven, all lots of seed have been treated in an electric oven provided with a constant temperature regulator. This oven which has been kept in a constant temperature incubator room during fall, winter and spring, and in the open laboratory during the summer, has maintained itself within one degree of the desired temperature. During the tests with seed of the 1923 and 1924 crops, this oven was equipped with a temperature recording device in order that the operator should be informed of any deviation from the desired temperature due to temporary failure of the heating current. All tests were discarded in which significant variations from the desired temperatures had occurred. A considerable number of tests made in the early part of this study had to be discarded because of an unobserved source of error which made them untrustworthy and which should be avoided by persons desiring to do work requiring the use of definite temperatures. This source of error arises from the fact that the difference in temperature between two positions on the same oven shelf may be surprisingly large. This became apparent when several lots of seeds which had been heated concurrently but for different periods of time on the same shelf of the oven gave unexpected differences in percentage of germination. The lotes heated for the shortest periods showed strong reduction in germination while those heated for the longest periods showed no reduction. When the temperatures of the different positions on the oven shelf were tested it was found that there are differences of as much as 10° C. between certain positions which are not more than 2 inches apart. This situation is represented in Figs. 1 and 2, which show the temperatures at certain indicated positions on the oven shelf when the oven is being operated at two different temperature levels. The small square indicates the locus of the oven thermometer. The circles show the temperatures found at the positions of the other thermometers when the oven thermometer stands at the temperature recorded in the square. The temperatures at one end and the back of the oven are too high while those at the other end and the front are too low. Obviously, when several lots of materials are being heated at the same time, and, presumably, at the same temperature, they are actually being subjected to temperatures which may differ sufficiently to introduce serious errors. After this variation in the temperature of different positions on the oven shelf was discovered, the different lots were heated singly and always in the same place and a thermometer was kept in direct contact with the seeds in order to make certain that they were actually being subjected to the temperature desired.

As cotton seed comes from the gin, it contains, as a rule, a goodly number of seeds possessing defective embryos and consequently incapable of germination. The greater part of these imperfect seeds may be recognized by their light weight, small size, and shrunken coats. In order to exclude undue variations in percentage of disease and germination which might arise from the presence of faulty seeds, only such seeds were selected as appeared capable of germination.

Unless otherwise specified, fifty seeds were used in each lot and the percentage of diseased seedlings is based on the number of seeds in each lot.

Except where otherwise specified, all germinations to determine the percentage of seeds carrying viable elements of the anthracnose fungus have been made by the method recommended by Barre.[13] Small squares of blotting paper were loosely placed in test tubes to make a column about 3-4 inch deep. This was moistened with tap water and the tubes were plugged and autoclaved at 15 pounds for 20 minutes. By use of long, sterile forceps, the seeds were placed one in each tube. The forceps were flamed after handling each seed as a precaution aganist carrying inoculum from seed to seed and thus increasing the percentage of infected seedlings. During the time required for each test the tubes containing the germinating seeds were kept in a covered, galvanized iron box containing an open vessel of water to prevent excessive drying while the seeds were sprouting.

The first records were usually taken after the seeds had been in the germinator 6 to 8 days. The ungerminated seeds and healthy seedlings were returned to the germinator for another period of 5 to 7 days after which the percentages of disease and germination were again recorded. Often the tubes were put back, and records taken after a third period, thus extending the time in the germinator to 20 or more days. Usually, germination is complete and the maximum of disease has developed by the 15th day. A microscopic examination of each diseased seedling was made in order to ascertain the cause of its death. In addition to the anthracnose organism, other fungi, especially Fusarium, Macrosporium and bacteria often attack the seedlings.

EXPERIMENTAL

As a preliminary study, it seemed desirable at the outset to determine, as nearly as possible the temperatures most favorable for germination of cotton seed, for growth of the anthracnose fungus and for development of the disease on the seedlings growing in test tubes. Accordingly, experiments were planned to test the effect of temperature on these processes.

EFFECT OF TEMPERATURE ON GROWTH OF GLOMERELLA GOSSYPII

Equal quantities of Czapek's nutrient solution[27] solidified with 1.5 per cent agar and adjusted to a reaction of pH 7.2 were poured in petri dishes of uniform diameter. After the agar had solidified the dishes were inoculated at the center with a loopful of a suspension of conidia in sterile water. Three of these cultures were then placed in each of four ovens which were adjusted to maintain temperatures of 21, 26, 30 and 34.5° C. Growth at the different temperatures was measured comparatively by determining the diameter of the colony at stated periods and by estimating the relative densities of the colonies formed and the abundance of the conidia produced. Chaudhuri[15] working with *Verticillium ablo-atrum* has shown that, while colony diameter is untrustworthy as a measure of the actual production of fungus material, it is satisfactory as a method of studying the relation of different temperatures to rate of growth in a medium of uniform composition and thickness.

TABLE II

Effect of Temperature on The Growth of *Glomerella Gossypii* on Nutrient Agar in Petri Dishes. (Increasing Abundance of Mycelium is Indicated by Number of † Marks. The Data Presented Represent The Average of Two Colonies in Each Case).

Exp. No.	Temp. Deg. C.	Diam. of Colony in mm.					Relative abundance at end of 4 Days		
		1 da.	2 da.	3 da.	4 da.	6 da.	Aerial Mycelium	Mycelium in Substratum	Conidia
1	21	No growth	5.5	16.5	26.5	----------	†	†	†
1	26	4.0	19.0	30.5	45.0	----------	†††	††	†††
1	30	5.5	19.5	30.0	44.5	----------	††††	†††	†
1	34.5	No growth	No growth	No growth	No growth	----------	----------	----------	----------
2	25	----------	14.5	25.0	41.0	61.0	†††	††	††
2	27.5	----------	15.0	28.5	40.0	58.0	†††	†††	†††
2	30.5	----------	16.5	27.5	38.5	57.0	††††	††††	†
2	33 0	----------	8.5	10.0	15.0	22.0	††††	††††	†

A study of Table II, which presents the data of this experiment (Exp. 1), shows that temperatures of 26 and 30° C. are about equally favorable for growth with a possible slight balance in favor of 30°. Growth was less abundant at 21° C. and nil at 34.5° C. A temperature of 26° was most favorable for conidial production. A repetition of this test using potato glucose agar gave results concordant with those of the first test. The results of a second experiment which was similar to the first in every detail except that the ovens were maintained at temperatures of 25, 27.5, 30.5, and 33° C. are also presented in Table II. This test (Exp. 2) shows that only negligible differences in growth occurred at temperatures of 25, 27.5 and 30.5° C. Much less growth took place at 33° C. Conidial formation was much better at the two lower temperatures.

The tests described above may be summarized by stating that except for the highest, growth occurred at all temperatures used and was greatest in the range from 25 to 30.5. It would seem that with a given lot of inoculated seed the disease should develop most rapidly and on the greatest number of seedlings within this range of temperature.

EFFECT OF TEMPERATURE ON GERMINATION OF COTTON SEED

In order to determine what are suitable temperatures for germination of cotton seed, the following experiment was performed. Six hundred seeds were shaken in a quantity of sterile tap water until the fuzz was thoroughly wetted through to the seed coat. These seeds were then divided into 6 lots of 100 seeds and each lot was placed on moist blotting paper in a separate, covered, glass germinator. The germinators were kept at temperatures of 15, 20, 25, 30, 35 and 40° C. The percentage of germination was ascertained at short intervals, and the hypocotyls were measured and the average length computed at the end of 64 hours.

TABLE III

Germination of Cotton Seeds at Different Temperatures (100 Seeds in Each Lot).

Lot No.	Temp. of Germinator Deg. C.	Per Cent of Germination After Hours Indicated					Av. Length of Radicle at end of 64 hrs. mm.	No. Seedlings Measured
		26	41	50	64	114		
1	15	0	0	1	31	48	None over 2 mm.	----------
2	20	2	61	89	95	96	16.8	96
3	25	11	63	85	94	94	17.1	97
4	30	64	97	99	99	----------	32.6	99
5	35	32	66	84	91	91	22.4	65
6	40	0	0	0	0	0	----------	----------

Table III, which presents the data obtained in this experiment, shows that, when judged by promptness and completeness, germination was decidedly most satisfactory in the lot held at 30° C. Very good and practically equal germination occurred in the two lots held at 20 and 25°, but these temperatures are obviously less favorable than 30°. At 35° germination was a little more prompt but less complete than at 20 and 25°. Sprouting was very slow at 15° and did not occur at all at 40°. When one compares the values found for the average length of the radicles, the different lots are again found to fall in the order given above indicating the relation of temperature to germination. The average length of the radicles of lot 4 was 32.6 mm., that of lot 5 was 22.4 mm., and that of lots 2 and 3 were 16.8 and 17.1 mm. The temperature of 35° is unfavorable for survival and continued growth of the seedling after germination. This is indicated by the fact that the seedlings die and decay much earlier at this than at lower temperatures. By the end of the 64 hour period in the germinator, 26 of the 91 seedlings had decayed to the extent that they were not used in obtaining the measurements.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF ANTHRACNOSE ON SEEDLINGS

Three tests were made to determine approximately the most favorable temperature for the development of anthracnose on cotton seedlings growing on moist blotting paper in test tubes. In the first test, disease-free seed of the Dixie variety were inoculated with a suspension of conidia and put into test tubes to germinate. One lot of 19 tubes was placed in each of four incubators set to run at temperatures of 20, 25.5, 30.5, and 35° C. In the second test, disease-free seedlings which had grown in test tubes for 13 days and which had reached a length of 5 to 6 cm. were inoculated by atomizing them with a suspension of conidia. They were then separated into 4 groups of 10 seedlings each and placed in incubators at temperatures noted above. In a third test, three lots of six-day old seedlings were atomized with a suspension of conidia and put to germinate at 18-25° (18° for 18 hours, 25° for 6 hours), 25.5, 30, and 33° C. Tables IV and V present the data derived from these three experiments.

TABLE IV

EFFECT OF TEMPERATURE ON THE DAILY DEVELOPMENT AND PERCENTAGE OF ANTHRACNOSE ON SEEDLINGS GROWING FROM SEEDS INOCULATED WITH A SUSPENSION OF CONIDIA OF *Glomerella Gossypii.*

		Days From Time Put to Germinate							
Exp. No.	Temp. Deg. C.	2	14	2	5	6	7	8	14
		Per Cent Germination		Per Cent Anthracnose					
1	20	60	60	0	0	0	10	60	60
1	25.5	60	60	0	30	60	60	60	60
1	30.5	70	70	0	70	70	70	70	70
1	35	90	90	0	0	0	0	0	0

TABLE V

EFFECT OF TEMPERATURE ON THE DAILY DEVELOPMENT AND PERCENTAGE OF ANTHRACNOSE ON SEEDLINGS ATOMIZED WITH A SUSPENSION OF CONIDIA OF *Glomerella Gossypii.*

Temp. Deg. C.	Experiment No. 2					Experiment No. 3			
	Days From Time Put to Germinate					Days From Time Put to Germinate			
	2	3	4	6	12	2	3	4	6
20	0	0	0	30	70	0[a]	0[a]	10[a]	60[a]
25.5	0	0	60	70	70	0	80	100	100
30	0	30	50	60	60	0	100	100	100
33	--------	--------	--------	--------	--------	0	60	60	90
35	0	0	0	0	0	--------	--------	--------	--------

[a] Temperature of this lot varied between 18 and 25° (18° for about 18 hours and 25° for 6 hours).

It is evident that the temperatures of 25 and 30° C. are very near the optimum for the development of anthracnose on seedlings grown under the conditions of these tests. The disease appeared on most of the seedlings incubated at 20° C. but at this temperature it developed more slowly and did not destroy the seedling as quickly as at 25 and 30° C. This slow development of the disease at 20° is probably correlated primarily with the less rapid growth of the causal organism at that temperature. At 35° C., a temperature which inhibits growth of the fungus, anthracnose failed to develop.

From the results of the several temperatures experiments described above, the following conclusions applying to the particular conditions of the tests may be set forth:

1. Temperatures of 25 and 30° are not only very favorable for germination of cotton seed and the subsequent growth of the seedlings but also closely approximate the optimum for growth in culture of the fungus causing cotton anthracnose and for the development of the disease.

2. While a temperature of 35° C. permits good germination of cotton seed, it inhibits the growth of the fungus which causes anthracnose and prevents the development of the disease.

3. At 20° germination of cotton seed and growth of the seedlings are good, but growth of the anthracnose fungus and the development of the disease are slower than at higher temperatures.

4. Temperatures between 25 and 30° C. are to be regarded as most favorable for the germination of cotton seed when it is desired to determine the percentage of infection from anthracnose by the method described in this paper. At these temperatures germination of the seeds is rapid and complete and timely development of anthracnose on inoculated seedlings is assured.

DRY HEAT TREATMENT OF COTTON SEED FOR CONTROL OF ANTHRACSE

EXPERIMENTS WITH SEED IN SMALL LOTS

The experimental work bearing directly on the control of cotton anthracnose was begun in a preliminary way by subjecting air dry cotton seed to temperatures of 70, 80, 90, and 100° C. and subsequently determining their ability to germinate. The data obtained in many of these preliminary tests later proved to be of doubtful value and had to be discarded, as noted above, because of the great variation in temperature which obtains between different positions on the same shelf of the oven used for heating the seeds. Inasmuch as several lots were usually heated concurrently, and no record of the position of the individual lot in the oven was kept, it was impossible, when this error was discovered, to tell, even by aid of the temperature map of the oven, the exact temperature to which a given lot had been subjected. Since differences of 10 to 15 degrees existed between lots heated at the same time, it was necessary to repeat many of the earlier tests, placing the seed only in positions known to have the temperatures indicated by the oven thermometer. Table VI gives the data obtained from such of the preliminary tests as are known to be trustworthy and from others made later in repetition of those which were discarded.

TABLE VI

Resistance of Air Dry Cotton Seed to Temperatures 70, 80. 90 and 100° C.

Exp. No.	Lot No.	Temp. Deg. C.	Time Hrs.	Per Cent Germination		Water Content of Seeds in Per Cent of Wet Wt.	Year When Seed Were Grown
				Treated	Untreated		
1	1	100	¼	0	100	----------------	1919
2	1	90	¼	0	100	----------------	1919
3	1	80	1	34	98	----------------	1921
3	2	80	4	24	98	----------------	1921
3	3	80	15	12	98	----------------	1921
4	1	80	1	5	98	----------------	1922
5	1	70	6	70	90	----------------	1919
5	2	70	24	40	90	----------------	1919
5	3	70	72	60	90	----------------	1919
6	1	70	24	98	92	8.3	1923
6	2	70	48	90	92	8.3	1923
7	1	70	24	80	97	14.3	1923
7	2	70	24	78	97	11.0	1923
8	1	70	24	73	71	7.98	1924
8	2	70	24	60	72	10.65	1924
8	3	70	24	49	77	13.22	1924

It early became evident that cotton seed in an air dry condition cannot endure the high temperatures used by Atanasoff and Johnson[2] and others in the treatment of cereal seeds. Air dry cotton seeds completely lost their power to germinate when heated at temperatures of 90 and 100° C. for periods as short as 15 minutes. The application of dry heat at 80° C. for one hour completely inhibited germination of seed of the 1919 crop and greatly reduced and prolonged germination of the 1921 and 1922 crops. Dry heat at 70° C. was endured for much longer periods without total loss of viability but even that temperature produced marked injury to certain lots of seed. In Experiment 5, in which three lots of seed of the 1919 crop were heated at 70° C. for 6, 24 and 72 hours, the treated seed yielded germination percentages of 70, 40 and 60 in comparison with 90 for the untreated check seeds. Other tests with seed of this crop yielded similar results. In a test (Exp. 6) with seeds of the 1923 crop whose water content had diminished to 8.3 per cent as a result of storage under laboratory conditions, two lots which had been heated for 24 and 48 hours gave respectively 98 and 90 per cent of germination in comparison with 92 per cent for the untreated seeds. In Experiment 7, two lots of seeds of the 1923 crop taken from the same container as those used in Experiment 6 were stored over water in a closed dish until they had absorbed sufficient vapor from the moist air to raise their water content to 11 and 14.3 per cent; then each lot was heated for 24 hours at 70°. A marked reduction in the percentage of germination resulted from the treatment. Seventy-eight and 80 per cent of the seed of the two treated lots and 97 per cent of the untreated lot germinated. In Experiment 8, three lots of seed of the 1924 crop were used. These seed originally came from the same bag but differed in water content as a result of different storage conditions for several days previous to the time of the heat treatment. Lot 1 was stored in an open dish in the laboratory, and underwent a marked reduction of its water content; lot 2 was taken from the center of a two bushel bag stored in the laboratory and had lost less water because of the bulk of the seed around it, and lot 3 was kept in a glass stoppered specimen jar. The three lots contained water amounting respectively to 7.98, 10.65 and 13.22 per cent of their wet weight, and after being heated at 70° for 24 hours, gave germination percentages of 73, 60 and 49 in comparison with 71, 72, and 77 for corresponding unheated lots. Seventy degrees appears to be slightly above the maximum temperature which cotton seed containing an amount of moisture normal to ordinary storage conditions can safely endure for any considerable period of time.

A number of tests were made to determine the amount of anthracnose which might develop on seedlings growing from seeds which had been heated at 70°. Table VII shows the results of some of these tests.

TABLE VII

Per Cent of Cotton Seedlings Infected With Anthracnose Growing From Seed Previously Heated at 70° C.

Exp. No.	Lot No.	Duration of Treatment, Hrs.	Per Cent Anthracnose	
			Treated Seed	Check
1	1	1	44	----------
2	1	24	2	20
2	2	48	1	20

When seed had been heated at 70° for one hour, 44 per cent of the seedlings developed anthracnose. After seed had been heated 24 hours, the percentage of seedlings which became diseased was reduced from 20 per cent in checks to 2 per cent in the treated. 48 hours at 70° C. did not suffice to completely free the seeds of anthracnose. Manifestly, a dry air temperature of 70° C. causes a great reduction in the amount of anthracnose appearing on seedlings from diseased seed, but fails to eliminate the disease within a period of time sufficiently short to obviate injury to seed containing a normal amount of water.

When it became apparent that the application of temperatures of 70, 80, 90 and 100° to cotton seed for periods of time sufficiently long to destroy the anthracnose fungus brought about marked reduction of germinative power of the seed, tests were made to determine whether decrease of the normal water content by desiccation at temperatures too low to be harmful would increase the resistance of the seeds to high temperatures. Accordingly, cotton seed having a normal air dry water content was stored over H_2SO_4 or CaO_2 in desiccators or dried in ovens at temperatures of 40, 50 or 60° C. for varying periods of time. After this preliminary desiccation, the seed were heated at temperatures of 60, 70, 80, 90 or 100° C. for certain periods of time. Such of these tests as deal only with water relations of treated seeds will be described and the results given in detail in a later section, Part II, of this paper. It will suffice to state at this point that drying of seeds at low temperatures enables them to endure for long periods of time temperatures which otherwise would have been quickly destructive. The following paragraps will be devoted to a consideration of the effect of this treatment on the viability of the seed-borne elements of the cotton anthracnose fungus. A large number of experiments have been made with seed grown in six different seasons. These tests are grouped, in accordance with the particular high temperature used and the results are set forth in appropriate tables.

Table VIII presents the data derived from a number of tests in which highly infected cotton seed were dried in ovens at 40-45° C. and subsequently heated at 60° C. for varying periods of time.

TABLE VIII

Per Cent of Anthracnose and Germination in Cotton Seed Which Has Been Subjected to Drying at 40-45° C. and Subsequent Heating at 60° C.*

Exp. No.	Lot No.	Time of Preliminary Drying at 40-45° C. Hours	Time of Heating at 60° C. Hours	Per Cent of Seeds Developing Diseased Seedlings		Per Cent of Seed Which Germinated	
				Treated	Untreated	Treated	Untreated
1	1	72	48	2	------------	86	------------
	2	72	72	0	------------	80	------------
	3	72	96	2	------------	82	------------
2	1	72	Not heated	14	40	96	96
	2	72	24	2	40	90	96
	3	72	48	4	40	92	96
	4	72	72	2	40	86	96
3	1	72	24	4	------------	96	------------
	2	72	48	2	------------	90	------------
	3	72	72	2	------------	94	------------
4	1	24	72	0	0	98	98
5	1	144	Not heated	4	40	92	96
	2	120	48	8	40	86	96

*Seed of the 1919 crop was used in all tests except Exp. 4 in which case seed was from the 1920 crop. In age the seed ranged from 10 months in Exp. 1 to 15 months in Exp. 3.

When comparison of the percentages of diseased seedlings derived from treated and untreated seed is made, it is obvious that this treatment greatly reduces but does not entirely eliminate seed-borne infection. Forty per cent of the seedlings from untreated seed developed anthracnose but not over 4 per cent from the treated seed became diseased. The preliminary drying at 45° reduced the number of diseased seedlings to 14 per cent in 72 hours and to 4 per cent in 144 hours. Strangely, heating at 60° for 24 hours seems as effective as the 72 and 96 hour period. Apparently the fungus in or on a very small percentage of the seeds is much more resistant than in the rest of the seeds. Experiment 4 shows that a preliminary drying for as short a time as 24 hours fully prepared the seed to endure 60° C. for 72 hours. A number of tests not here tabulated indicate that as a result of ageing, the disease had completely disappeared from the seed used in Experiment 4 by the time that experiment was made.

When it was learned that a temperature of 60° C. was not sufficiently high to kill all seed-borne infection in a reasonable time, a number of experiments were made using a temperature of 70° C. As before, the seed were given a preliminary drying at 40-45° C. Seed of the 1919, 1920 and 1921 crops, all heavily infected, were used in the experiments. Table IX sets forth the data pertaining to these tests.

TABLE IX

PER CENT OF ANTHRACNOSE AND GERMINATION IN COTTON SEED WHICH HAS BEEN SUBJECTED TO PRELIMINARY DRYING AT 40-55° C. AND SUBSEQUENT HEATING AT 70° C.*

Exp. No.	Lot No.	Temp. of Prelim. Drying Deg. C.	Time of Prelim. Drying Hours	Temp. of Heating Deg. C.	Time of Heating Hours	Per Cent of Seeds Developing Diseased Seedlings		Per Cent of Seeds Germinated	
						Treated	Untreated	Treated	Untreated
1	1	45-50	72	Not heated	Not heated	22	----------	84	----------
	2	45-50	72	70-75	24	6	----------	95	----------
	3	45-50	72	70-75	48	0	----------	100	----------
2	1	45-50	72	67-74	24	0	0	92	94
	2	45-50	72	67-74	48	4	0	96	94
	3	45-50	72	67-74	72	0	0	90	94
3	1	50-55	24	70-73	24	2	6	98	98
	2	50-55	24	70-73	48	0	6	100	98
	3	50-55	24	70-73	72	0	6	94	98
4	1	40-45	48	70	72	0	----------	90	----------
5	1	40-45	24	70	72	0	10	88	98
6	1	40-45	24	70-73	48	50	86	84	96
	2	40-45	24	70-73	72	6	86	92	96
7	1	40-45	24	70-73	72	24	64	92	94

*In Experiments 1 to 3, seed of 1919 crop was used; in Experiments 4 and 5, seed of 1920 crop; in Experiments 6 and 7, seed of 1921 crop. Experiments 1 to 3 were done in a gas oven. Seed used in Experiments 6 and 7 was 4 months old; that in Experiments 2 and 3 was 13½ months old.

It is evident that even after the seed have been dried at 45° C. a temperature of 70°C. is not sufficiently high to kill anthracnose in every instance even when the treatment is prolonged to 72 hours. All the tests except Experiments 6 and 7 were made at a time when the fungus was losing its viability from ageing as indicated by the behavior of check lots of untreated seed. Seed used in Experiments 1-5 was 10 to 16 months old when treated, while that of Experiments 6 and 7 was only 4 months old. The treatment proved to be much less effective with the young four months old seed of the 1921 crop than with the 10 to 16 months old seed of the 1919 and 1920 crops. Germination of treated and untreated seed are seen to compare favorably.

Rather than prolong the heating at 70° C. beyond a 72 hour period, it seemed better to try the effects of higher temperatures. In these, as in previous tests, the seeds were subjected to a preliminary desiccation before applying the higher temperature. The results of these tests, are presented in Table X.

TABLE X

Per Cent of Anthracnose and Germination in Cotton Seed Subjected to Preliminary Drying at 40 to 50° C. and Subsequent Heating at 80° C.*

Exp. No.	Lot No.	Temp. of Prelim. Drying Deg. C.	Time of Prelim. Drying Hours	Temp. of Heating Deg. C.	Time of Heating Hours	Per Cent of Seeds Developing Diseased Seedlings		Per Cent of Seeds Germinated	
						Treated	Untreated	Treated	Untreated
1	1	50	24	80	24	10	----------	96	----------
	2	50	24	80	48	12	----------	94	----------
2	1	40-45	48	80	48	22	----------	98	----------
	2	40-45	48	80	72	0	----------	94	----------
3	1	40-45	24	80	72	0	34	80	98
4	1	40-45	48	80	72	0	46	86	98
5	1	40-45	24	80	48	2	54	88	90
	2	40-45	24	80	72	0	54	88	90
6	1	40-45	24	80	48	4	64	94	94
	2	40-45	24	80	72	0	64	92	94
7	1	40-45	72	80	24	30	64	94	94
	2	40-45	72	80	48	2	64	96	94
8	1	40-45	48	80	48	2	----------	96	----------
9	1	50	12	80	72	2	----------	98	----------

*In Exp. 1-4, seed of the 1920 crop was used; in Exp. 5-8, seed of 1921 crop; in Exp. 9, seed of 1923 crop. The age of the seed used ranged from 3 to 10½ months.

At the temperature of 80° C. the seed-borne infection is seen to be greatly reduced by the end of 48 hours and completely devitalized in every test except one by the end of 72 hours. The 12-hour period of preliminary desiccation at 50° C. and the 24-hour period at 40-45° and 50° are as effective as the 48-hour period in preparing the seed to resist the high temperature of the different periods of heating. Prolonging the period of drying to 72 hours does not make it possible to shorten the period of heating to 48 hours.

In order to render this method of seed treatment more economical of time and heat, it seemed advisable to determine whether the effective period of heating at 80° C. namely, 72 hours, could be shortened by the use of a higher temperature. Accordingly, a number of tests were made in which infected seed were subjected to a treatment consisting of a preliminary desiccation of 40-45° C. or 50-53° C. immediately followed by a period of heating at 90° C. The results of these tests are presented in Table XI.

TABLE XI

Per Cent of Anthracnose and Germination in Cotton Seed Which Has Been Subjected to Preliminary Drying at 40 to 60° C. and Subsequent Heating at 90° C.*

Exp. No.	Lot No.	Preliminary Drying		Heating		Per Cent of Seeds Developed Diseased Seedlings		Per Cent Germination	
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Treated	Untreated	Treated	Untreated
1	1	40–45	48	90	48	0	34	56	98
	2	40–45	48	90	72	0	----------	22	----------
2	1	40–45	24	90	24	0	----------	54	----------
	2	40–45	24	90	48	0	----------	70	----------
3	1	40–45	24	90	6	30	----------	88	----------
	2	40–45	24	90	13½	0	----------	80	----------
4	1	50–53	24	90	10	14	----------	100	----------
	2	50–53	24	90	14	2	----------	94	----------
5	1	50–53	24	90	18	0	----------	82	----------
	2	50–53	24	90	24	0	----------	78	----------
6	1	50	24	Not heated	Not heated	90	88	94	90
	2	50	24	90	18	4	88	94	90
7	1	50	24	90	18	0†	----------	93	----------
8	1	50	24	90	24	0	----------	96	----------
9	1	40	24	90	18	0	16‡	36	92
	2	45	24	90	18	2	16	62	92
	3	50	24	90	18	0	16	96	92
	4	55	24	90	18	0	16	96	92
	5	60	24	90	18	0	16	98	92
10	1	50	18	90	24	0	42	68	96
	2	50	18	90	24	0	----------	88	----------
11	1	50	18	90	20	1†	82	97	100
12	1	50	12	90	24	2	----------	62	----------
	2	50	18	90	24	0	----------	92	----------
	3	50	24	90	24	0	----------	96	----------
	4	50	36	90	24	0	----------	96	----------

*Seed of 1920 crop was used in Exp. 1; 1921 crop in Exp. 2-5; 1922 crop in Exp. 6-9; 1923 crop in Exp. 11-12. The age of the seed varied from 1 to 12 months.

†100 seeds in this lot; 50 seeds in all others not so marked.

‡The seed used had been delinted for 30 min. in H_2SO_4.

A study of Table XI shows that the anthracnose fungus was killed in six and greatly reduced in amount in the remaining two of the eight tests in which the seed were heated at 90° C. for 18 hours. The disease was completely eliminated in eight of the nine tests in which seed were heated for 24 hours and failed to develop in any test when the seed had been treated for as long as 48 hours. A relatively large proportion of the infective material survived the 6 and 10-hour periods.

The low percentages of germination in Experiments 1, 2, and 9 (Table XI) show that a preliminary drying at 40-45° C. for 24 and 48 hours does not well prepare cotton seeds to endure a temperature of 90° C. for any considerable length of time. When they had been given a preliminary treatment at 50° for 24 hours (Exp. 5, 8, 9, and 12), little or no reduction in germination occurred as a result of heating at 90°.

In an effort to further reduce the time required for this treatment, a number of tests were made using 100° C. as the major temperature. Seeds harvested in 1921 and 1922 were dried for 24 hours at 40-45° C., or at 50° C. then heated at 100° for periods varying from 2 to 24 hours. The results of these tests are embodied in Table XII.

TABLE XII

Per Cent of Anthracnose and Germination in Cotton Seed Which Has Been Subjected to Preliminary Drying at 40-60° C. and Subsequent Heating at 100° C.*

Exp. No.	Lot No.	Preliminary Drying		Final Heating		Per Cent of Anthracnose		Per Cent Germination	
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Treated	Untreated	Treated	Untreated
1	1	40-45	24	100	11	0	----------	22	----------
	2	40-45	24	100	24	0	----------	50	----------
2	1	50	24	100	2	6	----------	32	----------
	2	50	24	100	4½	0	----------	6	----------
3	1	50	24	100	6	----------	----------	40	----------
4	1	50	24	100	12	0	38	68	92
	2	50	24	100	6	0	----------	58	----------
5	1	60	24	100	6	0	20	84	92

*Seed of the 1921 crop was used in Exp. 1-3; 1922 crop in Exp. 4; 1923 crop in Exp. 5.

A study of the data presented in this table shows that anthracnose developed on the seedlings in only one test, and in this instance, the seed had been heated only 2 hours. Inasmuch as germination was greatly reduced in all the tests after heating the seeds at 100° C., attempts to control anthracnose by use of this temperature were for a time discontinued. Several months later, after the encouraging results reported below had been obtained in the experiments in which temperatures at 60 and 95° were employed, another test was made in which 15 month old seed of the 1923 crop were heated at 100° for 6 hours. These seeds had been previously dried at 60° for 24 hours. Upon germination, none of the seedlings from treated seeds developed anthracnose (Table XIII, Exp. 5) while 20 per cent of those from untreated check seeds became diseased. Germination was appreciably but not excessively reduced by the treatment, 84 per cent of the heated seeds surviving the treatment.

The data embodied in Table XIII were obtained in a large number of tests in an effort to get better control of anthracnose than was obtained by heating seeds at 90° C. and still to obtain higher germination than seemed possible by the use of 100° C. In tests 1 to 6 the seeds were dried at 50° C. for periods of 12 to 36 hours, and in tests 7 to 9 the preliminary drying was done at 60° C. for 12 to 24 hours. The different lots were then heated at 95° C. for periods varying from 5 to 24 hours. In tests 8 and 9 the oven was provided with a recording instrument which made a continuous record of the temperature of the oven for the entire period of the treatment. The records of this instrument show that while the several lots of seed of these two experiments were being treated, no serious fluctuation of temperature occurred.

TABLE XIII

PER CENT OF ANTHRACNOSE AND GERMINATION IN COTTON SEED WHICH HAS BEEN SUBJECTED TO DRYING AT 50 TO 60° C. AND SUBSEQUENT HEATING AT 95° C.*

Exp. No.	Lot No.	Preliminary Drying		Subsequent Heating		Per Cent Anthracnose		Per Cent Germination	
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Treated	Untreated	Treated	Untreated
1	1	50	24	95	13	0	----------	90	----------
2	1	50	18	95	24	0	----------	64	98
	2	50	24	95	24	0	----------	72	98
3	1	50	18	95	12	0†	82	84	100
4	1	50	24	95	12	0	42	56	96
	2	50	24	95	12	0	----------	84	----------
5	1	50	24	95	7	0	----------	56	----------
	2	50	24	95	10	0	----------	84	----------
	3	50	24	95	5	0	----------	74	----------
	4	50	24	95	12	0	----------	46	----------
6	1	50	12	95	8	0	----------	38	----------
	2	50	18	95	8	0	----------	68	----------
	3	50	24	95	8	4	----------	80	----------
	4	50	36	95	9½	0	----------	96	----------
7	1	60	24	95	10½	0†	----------	95	----------
	2	60	24	95	12	0†	----------	98	----------
8	1	60	24	95	11½	0†	26	95	----------
	2	60	24	95	8	1†	----------	97	----------
9	1	60	6	95	12	0	----------	20	----------
	2	60	12	95	12	0	----------	86	----------
	3	60	18	95	12	0	----------	94	----------
	4	60	24	95	12	0	----------	96	----------

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*Seed of 1922 crop was used in Exp. 1 and 2; 1923 crop in Exp. 3-9. At the time of making the treatment the seed used in Exp. 1 and 2 was 12 and 13 months old, that in Exp. 3-9 varied in age from 2 to 6 months.

†100 seeds. 50 seeds in all lots not so marked.

As shown by table XIII anthracnose developed on seedlings from only two lots of treated seed, and in these two instances, namely Exp. 6, lot 3, and Exp. 8, lot 2, the seed had been heated for only 8 hours. In all the remaining lots of treated seed, some of which were heated for less than 8 hours, the viatality of the anthracnose fungus was completely destroyed while the untreated check lots yielded a high percentage of diseased seedlings.

The germination of the seed treated in Experiments 1-6 lot 3 was in most cases seriously reduced in comparison with that of the checks. Obviously, the heating of cotton seed containing a normal amount of moisture at 50° for 24 hours or less does not prepare the seed to withstand temperatures of 95° C. for any considerable period without marked loss of viability. When the seed are heated at 50° for 36 hours (Exp. 6, lot 4) or at 60° for 18 and 24 hours (Sxp. 7, 8, and 9, lots 3 and 4), excellent germination followed the subsequent treatment at 95° for 8 to 12 hours.

EXPERIMENTS WITH SEED IN BULK

As soon as it began to appear from the results of the tests made with seed in lots of 50 or 100 that control of anthracnose might be obtained by the application of dry heat for appropriate periods of time, experiments testing the efficiency of this method in the treatment of larger quantities of seed were begun. Use was made in a number of these tests of small gas and electric ovens such as are ordinarily found in plant pathological laboratories. In some of the tests the seed were spread out on the oven shelf; in others, they were held in shallow baskets made of window screen. When a 7 x 11 inch basket containing a quart of seed was heated on the shelf of an electric or gas oven of such size as to leave a margin of 2 to 3 inches for circulation of air, the seeds in the bottom of the pan always became very much hotter than those on top and were killed before those on top reached a temperature effective for destroying the anthracnose fungus.

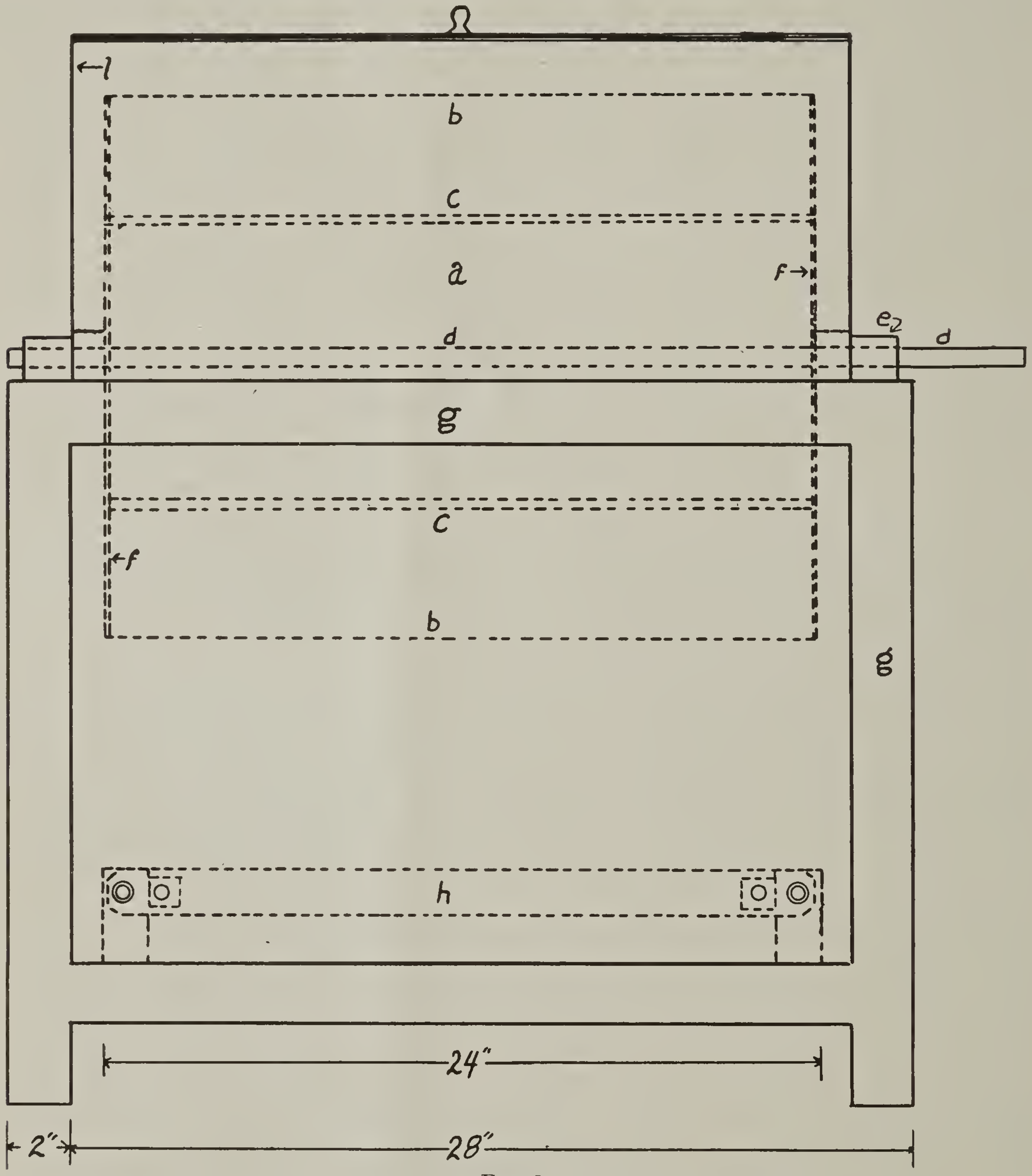
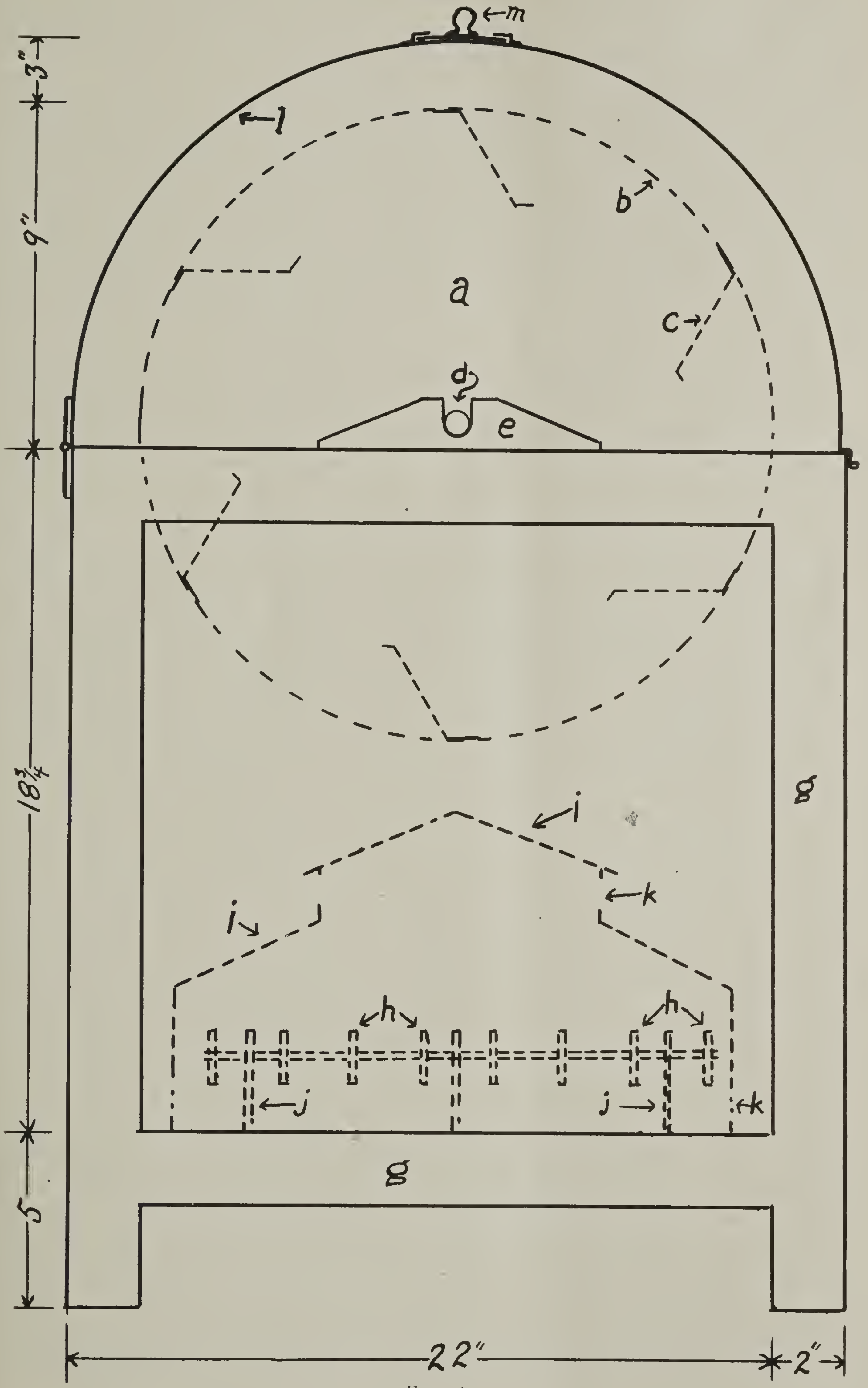


Fig 3

Figs. 3 and 4. Drawing to illustrate the general structure of the seed treating machine. Fig. 3, an end view; fig. 4, a side view from the front. A, seed drum; b, ⅛ inch mesh wire netting covering seed drum; c, metal shelves which serve to stir seed as drum rotates and to which the wire netting is attached; d, shaft which forms the axle of the seed drum and which is connected to the speed reducing gears; e, bearing for axle of seed drum; f, end of seed drum; g, angle iron frame; h, electric heaters; i, shield to prevent lint from falling on heaters; j, supports for heaters; k, apertures to facilitate circulation of air; l, galvanized iron hood; m, slide for regulating ventilation.

In other tests, a larger oven was used with a tier of 5 baskets 14 x 9 x 1½ inches, each containing a quart of seed. These baskets, kept apart by putting ½ inch wooden strips across the top were stacked in such a position as to leave a marginal space of 1 inch at the sides and 1½ inch


m
l
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c
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e
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i
k
i
h
h
j
j
k
g
3"
9"
18¾
5
22"
2"

FIG. 4.

at the ends for circulation of air. This too was unsatisfactory since great differences of temperature were found to prevail between the different baskets, the thermometers in the different baskets from the bottom upward showing 125, 110. 93, 81 and 75° C.

A larger oven was improvised from an old seed incubator which had shelves in the form of wire bottomed pans 21 inches square and 3 inches deep. About one peck of seed was heated at a time on one of these shelves, the seed being stirred at frequent intervals during the period of heating. Although by this procedure a much more uniform temperature could be maintained throughout the mass of seed, the results were unsatisfactory, both from the standpoint of regulation of the temperature and control of the anthracnose disease. Moreover, the method demanded the almost constant attention of the operator.

The difficulties which must ordinarily be overcome when one attempts to treat seed of any sort with dry heat are accentuated by the presence of the fuzz commonly found on cotton seed. When cotton seed is heated in bulk, the layers of fuzzy seed on the outside very effectively insulate the seed within the mass. When it was found that diseased seed could not be satisfactorily treated in bulk in ordinary gas and electric ovens, it was decided to build a special machine for the purpose. Accordingly, a machine which should keep the seed agitated while it is being heated was designed by the writer and built by Mr. V. W. Busbee, formerly of the Department of Mechanical Engineering. This seed treating machine is pictured in Plates I, II, and the construction is illustrated in Figs. 3 and 4. The rectangular body of the machine is 30 in. long, 18¾ in. tall and 22 inches wide and consists of a framework of heavy angle iron (g) welded into one piece. Asbestos board, 5/16 inch, is bolted to the iron framework to form the sides and ends, and heavy galvanized iron sheeting is used to form the bottom. One end is hinged to make a tightly fitting door. The corner angle irons extend 5 inches below the bottom to serve as legs. The semi-cylindrical hood or top (1) is composed of heavy sheet iron lined with sheet asbestos and is hinged so that it may be swung back out of the way when the machine is being loaded, unloaded or cleaned. A double row of ¾ inch holes in the bottom and a single row of 10 one-inch holes in the top provided with sliding shutters (m) serve to regulate the ventilation. The two upper end pieces of the angle iron frame carry bearings (e) for a shaft which supports a cylindrical drum (a) for holding and agitating the seeds while they are being heated. This drum is 24 inches long by 18 inches in diameter. The ends are made of heavy metal to which galvanized wire screening (b) having 8 meshes to the inch is fastened to form the curved sides. This screening is also fastened to flanges along the outer edges of 6 metal shelves (c) which are fastened between the ends of the cylinder in such a position as to lie in the plane of the radii of the cylinder. As the cylinder revolves; the shelves raise the seed from the bottom to near the top of the cylinder, and, while making this journey, the seed slide gradually off the shelf, falling to the bottom of the cylinder again. Thus the seeds are being constantly mixed and stirred and the hot air is kept circulating among them while the treatment is in progress. A small section of the side is so fitted as to be readily removed for loading and unloading the drum. The shaft (d) which passes along the longitudinal axis of the drum is fastened at one end to a system of reducing gears which in turn is connected by a belt to a small motor of ⅛ horsepower. By means of the reducing gear, the speed is reduced from 1800 revolutions per minute at the motor to about 1½ revolutions per minute at the drum containing the seeds.

The heat is supplied by 8 C-H electric space heaters* (h) placed in the bottom of the machine and directly under the drum for the seeds. In order to prevent lint and sand from accumulating on the heaters, a ventilated removale shield (k i) is inserted between the heaters and the seed in the drum. The temperature is regulated automatically by means of a thermostat which controls a circuit-breaker.

The sensitive element of the thermostat was made by firmly riveting together two strips of iron and zinc to make a tongue of metal 11 inches long and tapering gradually from 1 inch at the widest end to ⅜ inch at the tip. This strip of metal was bent into the shape of a narrow letter U with one side twice as long as the other. The end of the short arm was attached to a metal base and a point of platinum metal was attached to the tip of the long arm. A second platinum metal point was fastened into the tip end of an adjustable screw and the two parts bearing the metal points were set in such relative positions that when the temperature of the oven arose or fell, the two points made or broke contact respectively. The adjustable screw made it possible to increase or diminish the distance between the two points and thus to regulate the temperature of the oven. The thermostat is fastened to the inner surface of the front asbestos wall of the oven and suitably insulated wire connects its two parts to proper poles on the circuit breaker.

The circuit breaker (Fig. 5) is mounted on a slate base. It consists of two glass cups (e) containing a small amount of mercury and two electro magnets (d) capable of raising or lowering a U-shaped piece of metal (g). Tinned iron nails dipping into the cups of mercury are connected to the source of power and the bar of metal carries a short copper wire (f) to the ends of which other tinned iron nails are soldered. These nails are so adjusted in height above the mercury surface in the cups that when current is flowing through the thermostat they are held out of the mercury, but when the oven temperature falls and current ceases to flow through the thermostat, their points drop into the mercury cups, thus closing the heating circuit. A signal light burns when current is flowing through the heaters. Since the current which flows through the thermostat and operates the circuit breaker is taken from the same source as that which supplies the heaters, no batteries are needed. A condenser is connected across the contacts of the thermostat to reduce arcing at that point and consequent clatter of the circuit breaker. The motor which rotates the drum is operated independently of the heating circuit. The wiring diagram is shown in Fig. 5.

Thermometers inserted through the walls of the machine have made it possible to obtain a knowledge of the temperatures obtaining at different places in the machine while the seeds are being treated. In practice, it is

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*Manufactured by Cutler-Hammar Mfg. Co., New York.
Foot-note page 55.

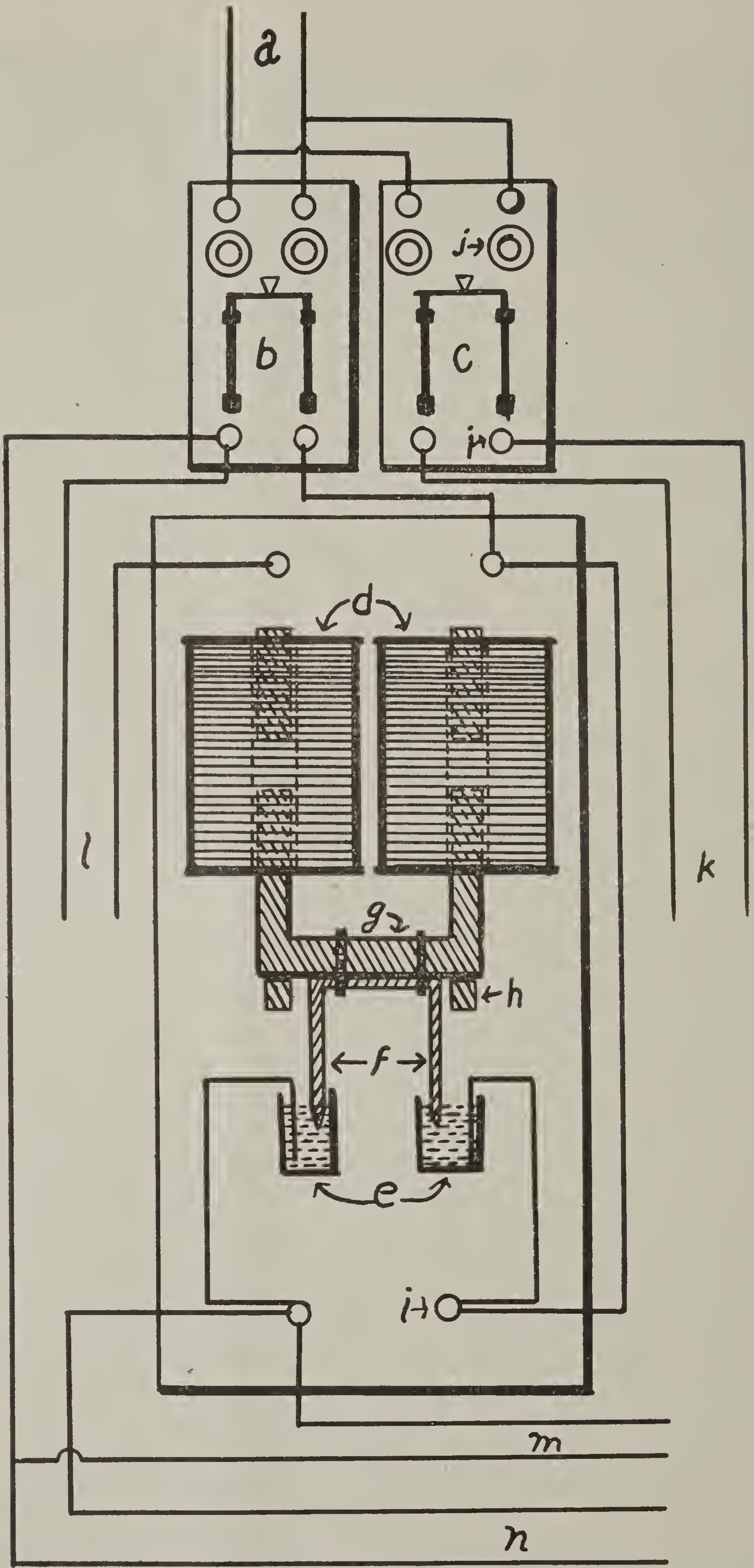

FIG. 5. The circuit breaker and wiring diagram; a, wires from the source of electrical supply; b, switch in heating circuit; c, switch in motor circuit; d, two electro magnets; e, glass cups containing mercury; f, conductor which connects the two mercury cups when current is flowing through heaters and which is raised out of mercury when the electro magnets raise the armature, g; h, electric heaters; i, shield, to prevent lint from falling on heaters; j, supports for heaters; k, apertures to facilitate circulation of air; l, galvanized iron hood; m, slide for regulating ventilation.

best to rotate the seed cylinder so that it comes up toward the front and descends at the back of the machine. The motion of the cylinder then directs the current of hot air toward the front of the machine where it strikes the thermostat. Since the seed are being carried toward the front of the machine and upward in the rotating drum, they are being heated by the same current of hot air which is playing upon the thermostat. At the level of the thermostat the temperature at the back of the oven is 10 to 15 degrees lower than at the front owing to the direction in which the air in the oven is circulating. The temperature at the top of the hood just above the cylinder is commonly 1 to 3 degrees C. lower than on the level of the thermostat in the front of the machine. In practice, the temperature shown by the thermometer inserted through the front right hand corner (Thermometer A) of the frame is taken to indicate the temperature of the seed: As indicated by this thermometer there is a variation of ½ to 1° C. between the temperatures at which the thermostat closes and breaks the heating circuit. After the heating circuit breaks, the temperature continues to rise 1 to 1½° C. and after the circuit closes, the temperature continues to fall 1 to 1½° C.; so including this lag there is a total variation of air temperature in the oven ranging from 2½ to 4° C. as indicated by thermometer A.

Some of the first lots of seed treated in this machine were ruined because of defective operation of the circuit breaker and thermostat. Modifications have been made which obviated these earlier defects so that the machine now operates in a very reliable manner. After the thermostat has been adjusted to give the temperature desired, it is necessary to visit the machine only occasionally to oil the bearings.

The total spatial capacity of the seed drum is 2.6 bu., after making liberal allowance for space occupied by the shelves which serve to mix the seed. If the quantity of seed treated at one time is not too large, the stirring of the seed by the rotation of the seed drum is very satisfactory. The efficiency of the machine in mixing the seed and thus assuring uniform heating was tested by embedding a small double-handful of seeds which had been stained red in the middle of the bulk of seeds to be treated and determining the time required for the red seeds to be distributed throughout the larger mass of seeds in the rotating drum. In one test, in which 18 pounds of dry seed (water content 8.5 per cent) were used, the red seeds had become within the lapse of 10 minutes uniformly distributed throughout the entire bulk of seeds in the revolving drum. In another similar test, 27 pounds of seed, a quantity which filled the drum very nearly half full, were used. After three minutes of rotation, the red seeds were present in about equal quantities in the middle region of each of the 6 compartments of the seed drum, and within 45 minutes, they had become distributed fairly uniformly throughout the entire bulk of seeds. Thus, both radial and longitudial mixing was found to be very satisfactory when the seed drum is slightly less than half full. Other tests showed that when the drum is more than half full, mixing is much less satisfactory, because, in a drum of the dimensions used in these tests, the seeds bulk up between the shelves and the axle and fail to slide off the shelves as the drum rotates. If the shelves were reduced in number from 6 to 4 or 3, the seeds should not so readily bulk between them and the stirring should very probably be satisfactory when the drum is considerably more than half full.

During the course of a treatment, considerable quantities of fuzz wear off the seeds and fall onto the heater shield; this should be cleaned off once or twice during the treatment. In one test in which 3600 grams of seed were treated, 22 grams of fuzz was collected during the treatment.

Certain workers in Egypt, namely, Story[29, 30] and Gough[21] have published descriptions of machines designed to treat cotton seed with dry heat as a means of combatting the pink boll worm. Since the larvæ of this insect are killed within a few minutes by heating infested seeds at temperatures of 60 or 65° continuous operation is possible. By means of belt or screw conveyors, the seeds in contact with hot steam conduits or hot air currents are carried slowly through the heating chamber, traversing the machine and reaching the exit in the course of about 10 minutes. These machines were unknown to the writer until more than two and one-half years after he had first used the one designed by himself for the control of anthracnose, and they are very different from it. Because of the higher temperature and much longer period of treatment required for control of anthracnose than for pink boll worm, it appears that the machines described by Story and Gough are not well adapted to the treatment required to destroy seed-borne elements of the cotton anthracnose fungus.

FIELD, GREENHOUSE AND GERMINATOR TESTS OF SEED TREATED IN BULK

While this investigation has been in progress, seed which had been treated in bulk have been planted in the field, in flats in the greenhouse and in test tubes in order to determine the effectiveness of the treatment in the control of anthracnose.

In 1921, diseased seed which had grown in the season of 1920 and which had been treated in an improvised gas oven as described on page were planted in small plots in fields on the farm of the Negro State Hospital at Goldsboro, N. C., and on the Coastal Plain Branch Station Farm at Willard, N. C. When these plots were visited on August 16, the cotton was in excellent condition, forming a good crop of bolls, but no anthracnose was found at either place. A second and final examination of these plots was made on September 24, 1921. No anthracnose was found on either treated or untreated plots on the farm at the Negro State Hospital. The complete absence of anthracnose from the untreated seed plot may be attributed to the dry weather which prevailed at this place during most of the fruiting season for cotton. At the Willard farm, where more humid conditions had prevailed, 8.6 per cent of the plants on the treated plot and 41.5 per cent of the plants on the untreated seed plot bore one or more bolls infected by the anthracnose fungus. These two plots which were on the same kind of land and had received the same cultural treatment were separated by 10 rows of corn. The results of this test indicate that the treatment given the seed had greatly reduced but had not completely eliminated the disease from the seed.

In 1922, diseased seed which had grown in 1921 and which had been treated in the new seed-treating machine described above were planted on the farms of the two branch stations near Willard and Rocky Mount, N. C. This land had not been in cotton for at least two years. The seed planted near Rocky Mount had been given a preliminary drying at an average temperature of 51° C. for 24 hours and were then heated at the average temperature of 90° C. for 22 hours. The treated seed plot consisted of 15 rows averaging 205 plants each and the untreated seed plot, which was located at a distance of 8 rods from the treated seed plot and separated from it by a corn field, consisted of 19 rows of 120 plants each. The seed planted near Willard had been dried at an average temperature of 50° C. for 24 hours, then heated at an average temperature of 80° for 70 hours. The treated plot consisted of 18 rows averaging 190 plants each and the untreated plot, 40 rods away comprised three rows about 80 rods long. At each farm the treated and untreated seed plots received the same fertilizer application and cultural treatment.

The tests on these two farms were examined twice during August. At the Willard station on August 9, an average of 4 to 5 per cent of the plants on the treated seed plot bore one or more bolls diseased with anthracnose while 17 per cent of the plants on the untreated plot bore one or more affected bolls. On August 30, 30.2 per cent, or 426, of the 1442 plants examined on the treated seed plot bore one or more diseased bolls while 45.7 per cent, or 86 out of 407, plants examined on the untreated seed plot bore diseased bolls. All the bolls on many plants of the check plot were infected while this was not the case on the treated seed plot. Although the treatment given the seed planted at Willard does not appear to have eliminated the disease, it did result in a later appearance and less damage from the disease in the field. At the farm near Rocky Mount, on August 9, only one diseased boll could be found by careful search over all the plants on the treated seed plot while an average of 2 to 3 per cent of the plants on the untreated seed plot bore bolls infected with anthracnose. On August 28, only 20 plants out of 2774 examined, a percentage of 0.7 per cent, on the treated seed plot were diseased; all but four of these were distributed in a small area as if they had become diseased as a result of secondary infections arising from the locus of a single diseased plant. Of the 777 plants examined, 178, or 22.9 per cent, on the untreated seed plot possessed one or more infected bolls.

While it appears that the anthracnose fungus was killed in nearly all the seeds of the treated lot, it also appears that the treatment was not sufficiently severe to completely eradicate the disease from the seed.

In 1923, diseased seed grown in the summer of 1922 was treated and planted at four different locations in the State, namely: on the farm of Mr. J. E. Dougherty, near New Bern, N. C., and on each of the three Branch Experiment Station farms near Willard, Rocky Mount and Statesville, N. C. The cotton on the farm of Mr. Dougherty exhibited, on August 14, vigorous vegetative growth, but nearly all the bolls had fallen off because of excessively wet weather and unusual boll weevil activity. No anthracnose was found on either the treated or untreated seed plots. The seed on the farm near Rocky Mount was planted late, which factor combined with excessively dry weather resulted in poor growth. When examined on September 18, no anthracnose was found on either the treated or untreated seed plots. The seed planted on the farm near Statesville had received treatment consisting of desiccation for 16 hours at 45-50° C. followed by heating for 24 hours at temperatures ranging between a minimum of 80° C. and a maximum of 90° C. When these test plots were examined on October 3, 6 per cent of the plants on the treated seed plot and 5 per cent on the untreated seed plot were found to be diseased. Slightly less than 1 per cent of the plants of the general crop of Early King cotton growing near by were diseased. The seed which were planted near Willard had been desiccated for 20 hours at 45-50° C. and heated for two 10-hour periods on two successive days at temperatures between a minimum of 70° C. and a maximum of 85° C. When these plots were visited on August 15, no anthracnose was found on either the treated or untreated seed plots, although the treated and untreated seed were planted through misunderstanding in adjoining plats. On September 18, 30 per cent of the plants both on the treated and on the untreated plots bore one or more infected bolls. Since anthracnose lesions appeared to have originated at punctures made by boll weevils, these insects were suspected of being responsible for this distribution of disease in both plots. Accordingly, a large number of them (99 in all) were collected in sterile tubes and carried back to the laboratory. Each weevil was shaken in 5cc. of water which was in turn poured into a plate of sterile nutrient agar. Colonies of the anthracnose developed in 17 per cent of the plates and in about one-third of these, anthracnose was the dominating flora. It is highly probable, therefore,

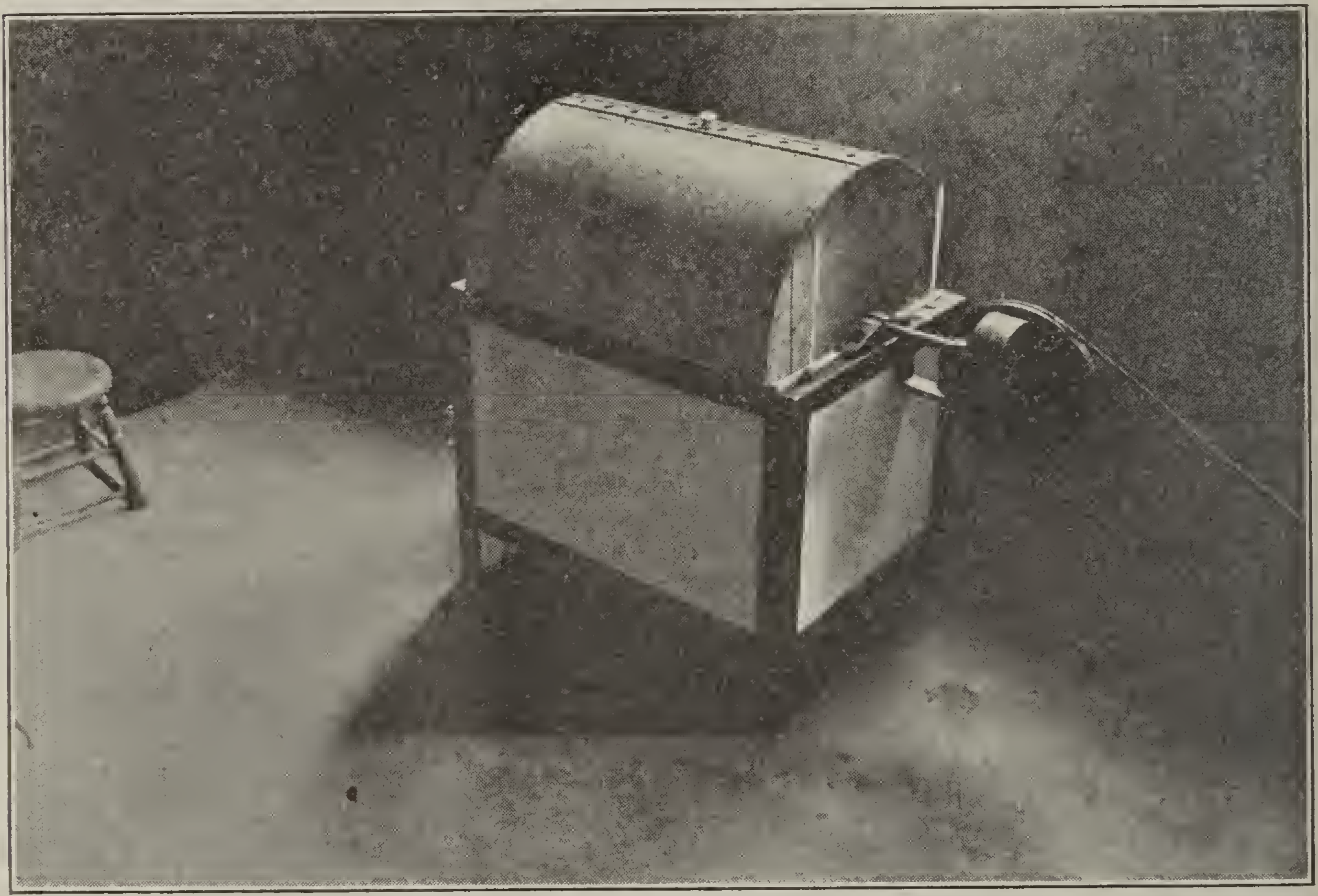

PLATE 1. The seed treating machine. Front and right end view showing the speed reducing gears and two thermometers in position.

that failure to find any difference in the amount of anthracnose on the treated and untreated plots in this test was due to the spread of spores of the anthracnose fungus from diseased to healthy plants by this insect.

A check on the results of the field trials on the farms near Statesville and Rocky Mount were made by plantings in the greenhouse. Four hundred seeds of each treated lot and 200 untreated seeds were chosen for these tests. By the end of 8 days, the germination was 92 and 96 per cent respectively, for the treated and 92 per cent for the untreated seed. By the seventeenth day, the percentage of anthracnose was 2 and 6 per cent respectively for the treated seed and 45 per cent for the untreated. The evidence from field and greenhouse trials is, therefore, in accord in showing that dry heat treatment greatly reduced the amount of viable anthracnose fungus present in these seed without impairing germination, but that the treatment was not sufficiently severe to completely destroy the fungus.

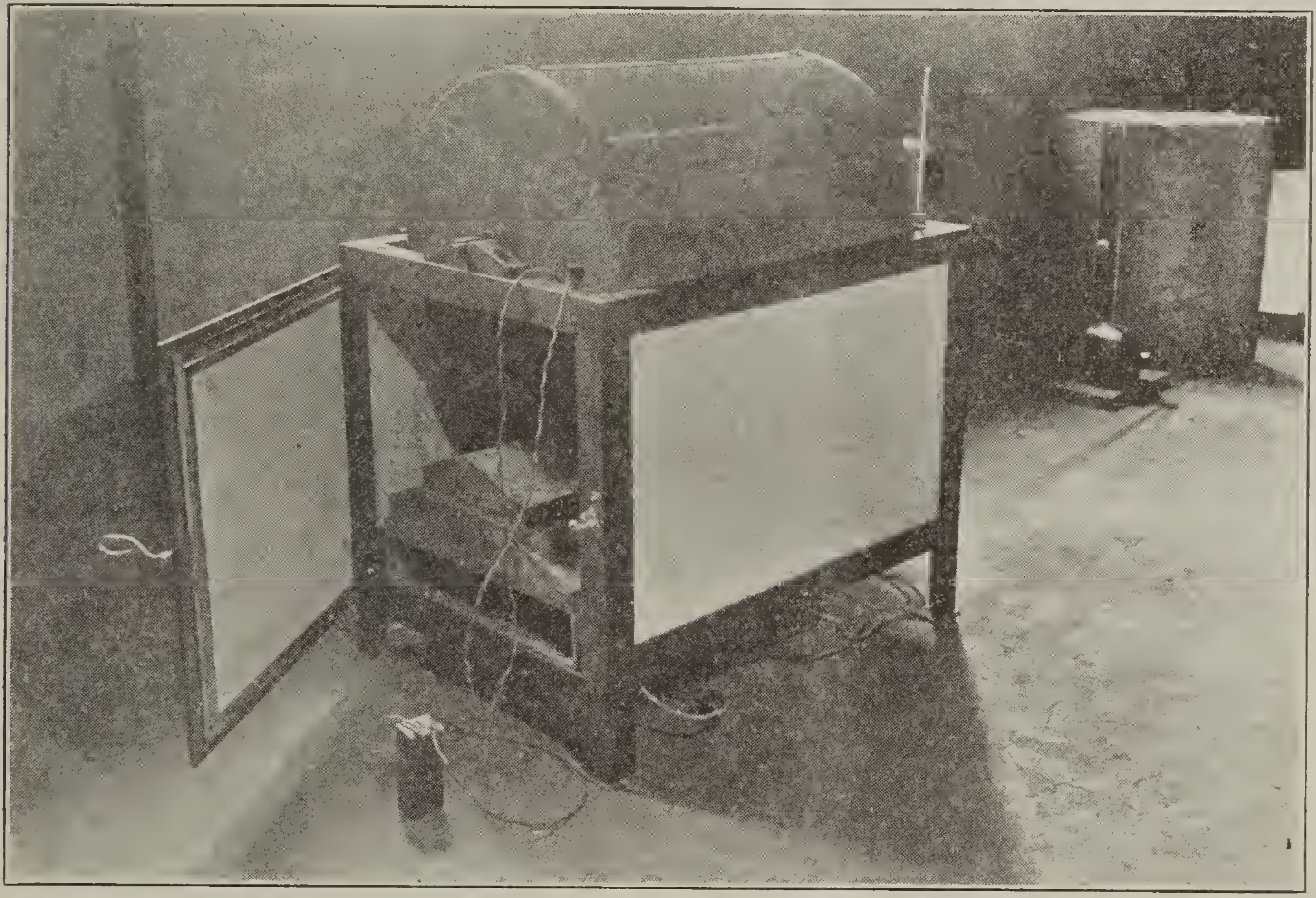

Plate 2. The seed treating machine. Front and left end view with hood removed and door open. The seed drum and the shield over the heaters are shown in position. The position of the thermostat which is here located inside the upper left corner, has been changed to the upper part of the front wall where it is more directly exposed to the hot air current which heats the seeds. The small condenser shown on the floor is connected across the points of the thermostat in order to minimize arcing and consequent clatter of the circuit breaker.

In still another test with seed of the 1922 crop, a quantity of seed was heated in the seed-treating machine at a temperature of 50° for 24 hours, then 100° C. for 12 hours. One thousand of these treated seed were planted in sand in flats in the greenhouse. Only 3 per cent of these seed germinated in 17 days making it evident that a temperature of 50° does not accomplish sufficient desiccation to enable the seed to withstand 100° for 12 hours. This result is in accord with results of tests on small lots of seed in the electric oven.

In 1924, highly infected seed which had grown in 1923 were treated. Inasmuch as the temperatures applied in previous years had failed to eradicate the disease and still leave the seed in a condition for good germination, higher temperatures were employed in the 1924 tests. In these tests, the seed were dried at 60-65° C. for 22 hours and heated at 95-100° C. for 12 hours. Because of the fact that the boll weevil now present in great numbers is known to carry spores of the cotton anthracnose fungus after visiting diseased bolls and may in this way transport the spores from diseased to healthy cotton over long distances; none of the seed treated in 1924 were planted in the field, but the efficiency of the treatment was tested by growing seedlings in sand in the greenhouse and in test tubes. Flats of sandy soil in the greenhouse were sterilized by drenching with formaldehyde and closely covering with paper for 2 days. The paper was then removed and the formaldehyde was allowed to evaporate over a period of 3 weeks. Three different tests of the lot of seed treated as described above were made. In test 1, 500 treated and 300 untreated seeds, and in test 2, 500 treated and 400 untreated seeds were planted in the flats. In test 3, 100 treated and 50 untreated seeds were germinated in test tubes. Test 1 was planted on June 16, test 2 on July 7, and test 3 on July 11. The seedlings in the greenhouse were kept well watered in order to encourage development of the disease on any of the seedlings which might be infected. Beginning on the eighth day and continuing at intervals of two days, all the seedlings from one or more of the flats were pulled. If lesions were present on cotyledons or stems, they were examined microscopically to determine whether they were caused by the anthracnose fungus.

TABLE XIV

PER CENT OF ANTHRACNOSE AND GERMINATION IN COTTON SEEDS TREATED IN THE SEED TREATING MACHINE. THE SEEDS WERE DRIED AT 60-65° C. FOR 22 HOURS, THEN HEATED AT 95-100° C. FOR 12 HOURS.
COOK COTTON OF 1923.

Test No.	How Tested	Per Cent of Anthracnose		Per Cent Germination		Number of Seeds Planted	
		Treated	Untreated	Treated	Untreated	Treated	Untreated
1	In flats in green-house	0	14.8%	92.4%	92.0%	500	300
2	In flats in green-house	0	2.6%	92.0%	96.7%	500	400
3	In test tubes	0	26.0%	99.0%	98.0%	100	50

Table XIV presents the summarized results of each of these tests. No anthracnose developed on any seedling which came from treated seed while as many as 26 per cent (test 3) of the seedlings growing from untreated seed became diseased. The percentage of germination of the treated seed exceeded that of the untreated in tests 1 and 3 and was slighly less than that of the check in test 2.

GENERAL CONCLUSIONS

Consideration of the experiments described above leads directly to the conclusion that the practical and complete control of cotton anthracnose in its seed-borne aspects can be obtained by the use of dry heat. The water content of the seeds which are to be heated is a factor of primary importance in determining the essential details of treatment. Control cannot be accomplished by the use of seed containing an amount of moisture normal to ordinary storage conditions, for dry heat at temperatures effective in killing the anthracnose fungus carried by such seed either completely destroys is viability or seriously reduces its germination. Air dry seed subjected to dry heat temperatures of 80, 90, 95 and 100° C. succumb before the anthracnose fungus is killed and at temperatures of 70° or less the vitality of the anthracnose fungus in not destroyed. However, after the moisture content of cotton seed has been sufficiently reduced by use of relatively low temperatures for appropriate periods of time, they may safely be treated at temperatures sufficiently high to prevent subsequent development of anthracnose on the seedlings. The vitality of the spores and mycelium of the anthracnose fungus carried on diseased cotton seed can be destroyed by the application of dry heat at temperatures of 95° C. for 12 hours or 100° C. for 6 hours. Temperatures of 80° for 72 hours, and 90° for 24 hours, accomplish the same result in all but a fraction of 1 per cent of the seeds, and it appears that prolonging the treatment at 80° and 90° to 96 and 48 hours respectively should destroy the seed-borne fungus in all the seeds. When cotton seed have been predried at proper temperatures for suitable periods of time, the application of dry heat at 80° for 72 hours, 90° for 24 hours or 95° for 12 hours, causes little or no reduction in percentage of germination. 100° C. for 6 hours causes a very considerable diminution in percentage of germination; however, the number of seeds which survive the treatment is sufficiently great to provide for an ample stand in the field.

The anthracnose fungus carried by a small proportion of the seeds is much more resistant than that carried by the majority of the seeds. Although 14 hours of heating at 90° reduced the anthracnose to 2 per cent, the fungus still survived in a small percentage of the seeds in 2 out of 8 tests in which heating continued for 18 hours and in 1 out of 9 tests in which heating lasted 24 hours. It may be that in the few seeds in which the fungus survived the relatively long periods of heating, the hyphæ of the fungus had penetrated deeply within the embryo of the seed and its protoplasm was by reason of its position embedded within other living tissue protected from the extreme desiccating and coagulating effects of the heat.

Dry heat treatment of cotton seed has certain obvious advantages over any method previously described for control of seed-borne anthracnose. It destroys all of the anthracnose fungus carried by diseased seed while chemical disinfectants and corrosives are only partially effective at best. It may be used in a much wider range of effective temperatures (80 to 100° C.) than is possible with the hot water method, and the period of treatment at any temperature within the effective range may be greatly prolonged without danger of reducing germination of the seed. It is an effective means by which the loss of time occasioned by the ageing of seed may be avoided; for diseased seed which has been properly treated by dry heat may safely be planted the season immediately following its production. Moreover, the seed grower need not assiduously avoid selection of seed from disease-free plants. The dry heat treatment for cotton anthracnose described by Lipscomb and Corley not only requires the reduction of the water content of the seed by strong preliminary desiccation before the application of the high temperature effecting control of the disease but also specifies that the seed be heated in a vacuum or in an inert gas, as nitrogen. The dry heat method described in this paper has the advantage of being less complicated in operation. The use of a vacuum or inert gas is not required, the seeds being heated in air after suitable preliminary desiccation.

By the use of a machine equipped with a proper stirring device, cotton seed may be successfully treated in bulk with dry heat. In the operation of the machine described in this paper, uniform heating is accomplished because of very thorough mixing of the seed, and for this reason, this type of machine is believed to be well adapted to the treatment of cotton seed with dry air at high temperatures. With this machine, the effective treatment for seed in bulk for control of the anthracnose disease without serious diminution of germinability consists of 20 to 24 hours of desiccation at 60 to 65° C., followed by 12 hours of heating at 95-100° C.

Practical application of the method of treatment herein described may be made by seed companies or associations interested in the production and sale of anthracnose-free seed, or by ginners who could treat seed for their patrons at a fixed price per bushel. By use of a machine of greater capacity than the one used in these tests, much larger quantities of seed can be treated at one time. A reliable source of heat is the prerequisite to successful operation. In large machines, steam heat could readily be substituted for electricity and the treatment could be made continuous by so constructing the machine that the hot air which has been used to heat the seed in the second stage of the treatment can be used to dry seed of a second and perhaps even a third lot which is at the same time passing through the first or drying stage, in which the aim is merely to reduce the water content of the seeds.

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II. GERMINATION OF COTTON SEED AND VIABILITY OF ANTHRACNOSE AS INFLUENCED BY DESICCATION AND BY STORAGE IN INERT GASES

The foregoing paper has been devoted to a method of treating cotton seed with dry heat for the purpose of destroying spores and mycelium of the anthracnose fungus. It is the purpose of this paper to describe certain other experiments which were conducted in order to study the germinability of cotton seeds which have been subjected to various treatments and to show the correlation between the water content and the resistance of cotton seeds to high temperatures and anthracnose control.

HISTORICAL

As early as 1834 Edward and Colin[21] tested the effect of heat on the vitality of the seeds of wheat, barley, rye, bean and flax. When heated in water for 15 minutes, all the seeds were killed by a temperature of 75° C. and the upper temperature limit at which the seeds were not injuriously affected was found to be 50° C. When the seeds were heated in water vapor, all were killed at 75° C., but at 62° C. little harm was done, whereas when heated for 15 minutes in dry air at 75° C. many were capable of germination. It was thus found that the medium in which the seeds were heated determined the temperatures that they were able to endure without loss of germinative power.

In 1879, Noble[44] heated wheat, rye, and oats seeds having a water content had been heated for one hour in water at 55° C. and that all were killed when heated at 60° C. When heated in dry air, the seeds endured temperatures of 65° C. for 4 hours, 85° C. for 30 minutes, and 90° C. for 15 minutes, but all were killed in 15 minutes by a temperature of 95° C., and in 10 minutes by a temperature of 100° C.

In 1863, Haberlandt[29] heated 88 species of dry seeds in dry air at 100° C. for 48 hours. Twelve species entirely lost their power to germinate, 12 species suffered partial killing, and 64 species gave full germination, many of this last group, however, showing marked retardation. When the same kinds of seeds were heated at 87.5° C. for 48 hours, only 2 species completely lost their power to germinate, 17 species were partially killed, 34 species showed marked retardation of germination, and 35 species manifested an acceleration of germination. No injury was observed to result from an application of a temperature of 56-57° for 48 hours.

Haberlandt[29] also studied the effect of inbibed water on the viability of seeds of rye, corn, flax and peas. Air dried seed and seeds which had been allowed to swell in water at a temperature of 12 to 15° C. for 24 hours were heated in water at a temperature of 30-55° C. for periods of 5-10 hours. With every species, the soaked seeds proved the more susceptible to the injurious effects of heat, as evidenced by the greater reduction of total percentage of germination and more pronounced retardation in rate of germination.

In 1897, Noble[44] heated wheat rye, and oat seeds having a water content of 12 to 13 per cent at temperatures of 40 to 100° C. in dry air. Wheat and rye suffered no destruction of vitality when heated at 80° C. for a period of time sufficiently long to reduce the water content to 1 or 2 per cent. Heating at 90° C. for 2 days, whereby the water content was reduced to less than 1 per cent, first lessened the power of germination, and a temperature of 100° C. for 24 hours almost completely destroyed it. Oats was reported to be more susceptible to high temperatures than wheat or rye. Drying oats for 3 days at 50° and 60° strongly retarded and delayed germination.

In 1899, Jodin[38] showed that the water content of seeds profoundly influences the time which they can survive high temperatures. This worker heated wheat seed in a vacuum to a temperature of 100° C. without destroying its power to germinate. Pea and cress seeds were killed when heated at 98° C. for 6 hours, but when seed from the same lots were heated at 60° C. for 24 hours, then at 98° C. 30 per cent of the peas and 60 per cent of the cress still germinated. When seeds were heated in sealed tubes at 40° C., they were killed in 20 days, but, when the seeds were sealed up with CaO, germination was not reduced by heating for 206 days at 40° C.

Dixon ([16], [17]) in 1901 and 1903 found that seeds of many species which had been dried over H_2SO_4 or in an oven which was slowly raised to a temperature of 90° could thereafter endure, without complete loss of ability to germinate, temperatures ranging from 100 to 121° C. for a period of one hour. He found that *Avena sativa* and *Hordeum distichum* withstood a temperature of 118° C. and *Medicago sativa* survived 121° C. although exposure at temperatures near the maximum increased the time needed for germination.

In 1917, Wagonner[61] published the results of experiments on the effect of different temperatures on the viability of radish seeds whose water content varied from 0.4 per cent to 71 per cent. The temperatures employed ranged from 50 to 125° C. and the seed were heated in stoppered flasks, immersed in a water bath. When heated at 60° C., those seeds whose initial

Plate 4. Cleveland big boll cotton of the 1924 crop growing in the field after a dry heat treatment which consisted of preliminary desiccation at 60-65° for 22 hours and subsequent heating at 95-100° C. for 12 hours. This seed was of poor quality to begin with because of unfavorable weather at the time it was maturing, and the treatment reduced the germination from 69 per cent to 60 per cent. The seed was planted on April 23 and the photograph was taken on May 21. During most of this time, the weather was very unfavorable for growth of cotton. From 50 to 75 per cent of the seedlings of the general crop died largely from an attack of anthracnose following cold weather and the greater portion of the acreage had to be replanted. In the treated plot sickly seedlings were extremely rare and in no case could anthracnose spores be found on the few which were present while anthracnose spores were abundant on 90 to 100 per cent of the sickly seedlings found on untreated plots. Although the seedlings on the rteated plots were making but little growth they were sturdy, remarkably healthy and possessed strikingly good color in comparison with seedings from untreated seed on nearby plots.

water content was 45 per cent or more were killed whereas those containing only 4 per cent of water suffered no reduction in percentage of germination. Complete loss of viability occurred when seeds containing 4 per cent of water were heated at 100° C. while on the contrary seeds containing only 0.4 per cent of moisture gave as high a percentage of germination as untreated seed. A small percentage of the seeds containing 0.4 per cent water survived a temperature of 123° C. Thus the resistance of radish seeds to heat was found to bear an inverse relation to initial water content at the time of heating. Temperatures approaching the maximum for the seed of a given water content caused more or less pronounced retardation of germination.

Burgess[6] in 1919 published data showing the percentage of germination of certain agricultural seeds which had been subjected in an air dry condition to high temperatures. Wheat and rye were killed by an exposure of one hour to 248° F. but gave respectively 55 per cent and 78 per cent germination when heated for 3 hours at 230° F. Ninety-eight per cent of oats and 2 per cent of cowpea seeds germinated after 5 hours of heating at 194° F. Soybeans gave perfect germination after exposure for one hour at 194° F., and soybeans, garden beans and cowpea seeds gave respectively 100 per cent, 92 per cent and 78 per cent germination after subjection for 3 hours to heat at 176° F.

In 1920, Saillard[47] subjected sugarbeet seed of three grades designated "very good," "good" and "poor," which gave before treatment, 98, 87 and 36 per cent germination respectively, to dry heat at temperatures ranging from 60 to 105° C. After treatment at 80° C. the respective percentages of germination for the three lots was 97, 86, and 9 per cent; after treatment at 90° C., 95, 75 and 2; and after treatment at 100° C., 80, 46 and 0. Of the "very good" seeds 3 per cent survived the treatment at 105° C.

In 1922, Gain[22] published the results of experiments with sunflower seeds. He found that by carefully drying and heating the seeds for suitable periods of time at temperatures which were raised by successive steps, many of the seeds could be prepared to endure temperatures as high as 150° C. After similar treatment, seeds of a number of other plants were found to endure temperatures of 110 to 155° C. without complete loss of power to germinate. Similar experiments by Sigalas and Marneffe[51] confirm the work of Gain.

In addition to the workers whose papers are reviewed in the above paragraphs, others as Groves,[26] Crocker and Groves,[10] Goodspeed,[25] Harrington and Crocker,[31] Honing,[35] Hausdorfer,[32] Schneider-Orelli,[49] Neuberger,[45] Just,[39] and Schubert[50] have published from time to time the results of studies on the effect of desiccation and the application of heat on the viability, longevity and germination of seeds. In general, these workers have found that air dry seeds are much more resistant to dry than to moist heat and that preliminary desiccation over chemicals or at low temperatures greatly increases the ability of seeds to withstand exposure to high temperatures.

Few workers have published any data showing the effect of dry heat on cotton seed. The publications of Story, [53], [54] Gough[24] and Lipscomb and Corley[43] have been cited in an earlier part of this paper. Gough, using a machine designed for treatment of cotton seed to kill the pink boll worm found that, when the seed remained in the machine for nine minutes and the temperature of the seed did not exceed 51° C., very little reduction of germination resulted, but injury was marked at 61° C. and very severe at 72° C. Lipscomb and Corley found that ,while cotton seeds in an air dry condition are quickly destroyed by a temperature of 100° C., they may endure this temperature for 26 hours without loss of viability if they are first thoroughly dried and then heated in a vacuum or in an inert atmosphere as nitrogen.

EXPERIMENTAL

A number of experiments were performed in order to determine the temperature and period of time most suitable and practical for drying cotton seed in order to condition them to withstand the higher temperatures required for killing the anthracnose fungus. In certain of these experiments the extent of dehydration was measured by determining the proportion of water lost during the treatment. In all these tests, precaution was taken to have the separate lots reasonably uniform by first selecting and thoroughly mixing enough seeds for the entire experiment then mechanically dividing the heap into the number of lots required. Each lot was dried separately in one electric oven and heated in another, so it was not necessary to change the adjustment of the oven thermostat during the course of an experiment.

Table I gives the data of Experiment 1 in which 5 lots of seed were dried at temperatures of 40, 45, 50, 55 and 60° C. for 24 hours and afterward heated at 90° for 18 hours. The percentage of germination was recorded at the end of 2, 3, 4, 5, 6, 9 and 14 days.

TABLE I

EFFECT OF DESICCATION AT TEMPERATURES OF 40, 45, 50, 55, AND 60° C. FOR 24 HOURS AS A MEANS OF PREPARING COTTON SEED TO WITHSTAND A TEMPERATURE OF 90° C. FOR 18 HOURS. 50 SEEDS IN EACH LOT. CROP OF 1922.

Exp. No.	Lot No.	Preliminary Drying		Subsequent Heating		Per Cent of Germination						
						Days in Germinator						
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	2	3	4	5	6	9	14
1	1	40	24	90	18	0	4	16	22	28	28	36
	2	45	24	90	18	0	28	60	62	62	62	62
	3	50	24	90	18	4	74	92	----	96	96	96
	4	55	24	90	18	2	76	96	----	96	96	96
	5	60	24	90	18	2	66	96	----	96	98	98

Lots 1 and 2, which had been dried at 40 and 45° C. respectively showed marked diminution in both the rate and final percentage of germination. Temperatures of 40 and 45° C., when applied for periods not longer than 24 hours, do not condition cotton seed to endure without injury to germination the temperature of 90° C. for 18 hours. For this purpose it is necessary to use a temperature of 50° C. or above. In all lots which were dried at 50° C. or above, germination was prompt and entirely satisfactory in amount.

In Experiment 2, the results of which are set forth in Table II, the temperature of drying was kept the same for all lots but the period of heating was varied. Four lots were dried at 50° C. for 12, 18, 24 and 36 hours, and subsequently heated at 90° C. for 24 hours. The percentage of germination was recorded at the end of 7, 13 and 19 days and the radicles of all the seedlings which had germinated and remained healthy were measured at the end of seven days. From these measurements the average length of the seedlings in each lot was determined and recorded, and the measurements are arranged in groups on the basis of the length of the seedlings and recorded in the table in order to show the correlation which exists between the period of drying and the rate of germination.

The percentage of germination of the lots treated for 12, 18, 24, and 36 hours, was 62, 92, 96, and 96 respectively on the 19th day, and the average length of the radicles was 20.6, 46.7, 44.6, and 51.7 mm. respectively on the 7th day. The correlation table shows that germination of lot 1 was not only greatly reduced in amount but also markedly diminished in rate, for the large groups of seedlings in this lot are to the left of the table and in the classes characterized by short radicles; while in lots 2, 3, and 4, the large groups are at the right side of the table and in classes characterized by long radicles. The facts of the reduced amount and retarded rate of germination and the short average length of the seedlings in lot 1 point very definitely to the conclusion that when seeds are to be heated at 90° for 24 hours, the 12-hour period of drying at 50° C. is too short. The 18-hour period is much better and the 24 and 36 hour periods are entirely satisfactory.

Experiment 3, the results of which are also presented in table II, is like experiment 2 in every detail except that after preliminary drying at 50° C. the seeds were heated at 95° C. for 8 hours. In this experiment, the germination of the 4 lots dried for 12, 18, 24 and 36 hours, was 38, 68, 90, and 96 per cent respectively and the average length of the radicles of the seedlings increased with increase in length of the period of drying. In lots 1, 2, and 3, germination was both reduced in amount and retarded in rate and only in lot 4, which was dried for 36 hours, did germination occur without evidence of injury. It is evident from the results obtained from this experiment that when seeds are to be heated at 95° C. for as long as 8 hours, the 12, 18 and 24-hour periods of drying at 50° C. do not give sufficient protection while the 36-hour period affords ample defense against the ordinarily injurious effects of the higher temperature.

Since in experiments 1, 2, and 3 described above the protection given the seeds by the preliminary drying increased within limits with increase in both temperature and time of drying, it semed reasonable to assume that the increased protection is due primarily to the loss of water which occurs during the period of preliminary drying. In order to test this matter, certain experiments were made in which the amount of water lost was determined by weighing the seeds accurately at different stages in the course of the treatment. The water loss during the period of the preliminary drying and during the time of heating at 95° C. was determined for each lot, and is given in Table III as percentage of the original air dry weight of the seeds. The total water content of a lot of seeds comparable in every way to the treated lots was determined by heating 100 seeds to constant weight at a temperature of 103-105° C., and this value is recorded in the table also as percentage of the air dry weight of the seeds. Using the values obtained for the water content of the air dry seeds and for the

TABLE II

Effect of Drying at 50° C. For Different Periods in Conditioning Cotton Seed to Withstand Temperatures of 90 and 95° C. 50 Seeds in Each Lot. Cook Cotton of 1923 Crop.

Exp. No..	Lot No.	Preliminary Drying		Heating		Per Cent Germination			Correlation of Period of Preliminary Drying With Effectiveness thereof*										
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Days			Group Characteristics, Length and Number of Seedlings in Each									Average Length of Seedlings mm.	Number Seedlings Measured
						7	13	19	0–9 mm.	10–19 mm.	20–29 mm.	30–39 mm.	40–49 mm.	50–59 mm.	60–69 mm.	70–79 mm.	80–89 mm.		
2	1	50	12	90	24	50	58	62	9	6	2	3	2	2	1	0	0	20.6	25
	2	50	18	90	24	92	92	92	5	2	4	3	7	10	10	4	1	46.7	46
	3	50	24	90	24	96	96	96	3	3	5	3	12	10	9	3	0	44.6	48
	4	50	36	90	24	96	96	96	2	2	3	8	4	7	10	8	4	51.7	48
3	1	50	12	95	8	28	38	38	4	3	5	0	0	0	1	0	0	18.0	13
	2	50	18	95	8	64	66	68	10	3	4	4	5	3	0	0	1	25.6	30
	3	50	24	95	8	74	80	80	10	4	5	1	8	6	1	1	1	31.0	37
	4	50	36	95	8†	96	96	96	0	1	2	4	14	14	8	4	0	50.4	48

*As indicated by the average length of the seedling radicles and by the number of seedlings in the different growth groups characterized by length at the end of 7 days in the germinator.

†This lot actually ran 9½ hours at 95° C.

water lost by the air dry seeds during the period of preliminary drying, the water remaining in the seeds at the end of the period of preliminary drying was calculated and is given in the table as percentage of the oven dry weight of the seeds. In Experiment 4 the preliminary drying was done at 60° C. for periods of 12, 18, and 24 hours, and in experiment 5 at 50° C. for 24 and 36 hours. In experiment 6 the different lots were dried at 40, 45, 50. 55, and 60° C. for a uniform period of 24 hours. In all three experiments the final treatment was done at 95° C. for 12 hours. After the heat treatments the seeds were germinated between blotters and the percentage of seeds which sprouted was recorded at intervals in order to get some idea of the rate of germination. Also the average length of the radicles of the seedlings which were not attacked by bacteria or fungi was determined at the end of the 5-day period in the germinator.

All the seeds dried at 40° C. and all except 5 dried at 45° C. were killed by the subsequent treatment at 95°. During the preliminary period of drying these seeds lost water equivalent to 3.5 per cent or less of their air dry weight and retained an amount of water equal to 5.54 per cent or more of their oven dry weight. The two lots which were desiccated at 50° for 24 hours and the two lots dried at 60° for 12 hours were retarded in rate and greatly reduced in amount of germination. These seeds lost more water during the period of preliminary drying than those which were dried at 40 and 45°, but the amount retained was still too great to enable the seeds to withstand well the treatment at 95°. The seeds which were dried at 60° for 18 and 24 hours suffered no ill effects from the treatment at 95°, but on the contrary germination was as complete and somewhat more prompt than in untreated check seeds. During the desiccation at 60° these seeds lost water equal to 5.24 per cent or more of their air dry weight and retained an amount equivalent to 3.36 per cent or less of their oven dry weight. In no test in which the final treatment of the seeds was at 95° for 12 hours and in which the seeds lost as much as 5.24 per cent of their weight during the period of preliminary drying did the treatment at 95° cause pronounced loss of viability. The lot which was dried at 55° and which lost 5.51 per cent of its weight appears to have suffered slight reduction in amount of germination. Aside from this one lot, the seeds which lost as much as 5.24 per cent of their weight germinated more promptly and as completely as untreated seed. As a result of the study made in experiments 4, 5, and 6, a statement expressing the relation between water loss and resistance to high temperature may be formulated to the effect that cotton seeds may be conditioned to withstand a temperature of 95° C. for 12 hours by desiccating them at low temperatures until they have lost not less than 5.24 per cent of their dry weight. Obviously, this statement can apply only to seeds whose water content is not much greater than 8.33 per cent or 8.57 per cent of their air dry weight; for with seeds containing more than this amount of water, the removal of 5.24 per cent of their weight would still leave the living protoplasm too strongly hydrated to long withstand a temperature of 95°, while, on the other hand, seeds containing less than 8.33 per cent of water can obviously be conditioned to withstand 95° for 12 hours by removal of water equivalent to less than 5.24 per cent of their dry weight. A much more broadly applicable criterion of the ability of seeds

TABLE III

WATER LOSS AND WATER CONTENT AS RELATED TO DIFFERENT PERIODS OF PRELIMINARY DESICCATION AND TO GERMINATION OF COTTON SEEDS SUBSEQUENTLY HEATED AT 95° C. FOR 12 HOURS. 100 SEEDS IN EACH LOT. COOK COTTON OF 1923 CROP.

Exp. No.	Lot No.	Preliminary Treatment		Final Treatment		Per Cent Germination						Average Length of Radicle at end of 5 days mm.	Number Seedlings Measured	Water Content in Per Cent †	Per Cent Water Loss by Prelim. Treatment †	Per Cent Water in Seeds After Prelim. Drying *
						Time in Germinator										
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	68 Hours	72 Hours	91 Hours	5 Days	7 Days	9 Days					
4	1	60	12	95	12	7	--------	53	66	70	71	9.5	51	8.33	4.58	4.11
	2	60	18	95	12	58	--------	96	96	96	96	--------	--------	8.33	5.38	3.19
	3	60	24	95	12	66	--------	97	97	97	97	16.4	96	8.33	5.68	2.88
	4	Check	Untreated			32	--------	85	91	96	96	12.6	85	8.33	--------	--------
	5	60	12	95	12	25	--------	76	80	81	82	--------	--------	8.33	4.88	3.74
	6	60	18	95	12	68	--------	98	98	98	98	--------	--------	8.33	5.24	3.36
	7	60	24	95	12	52	--------	95	95	95	95	--------	--------	8.33	5.60	3.00
5	1	50	36	95	12	43	--------	87	92	93	93	13.3	86	8.33	5.01	3.62
	2	50	24	95	12	14	--------	47	61	66	66	--------	--------	8.33	4.55	4.11
6	1	40	24	95	12	0	0	0	0	0	--------	--------	--------	8.57	2.68	6.43
	2	45	24	95	12	0	0	1	2	5	--------	--------	--------	8.57	3.50	5.54
	3	50	24	95	12	4	21	46	56	64	--------	12.6	48	8.57	4.99	3.90
	4	55	24	95	12	13	53	83	89	90	--------	13.9	78	8.57	5.51	3.34
	5	60	24	95	12	16	59	96	99	99	--------	15.5	94	8.57	6.08	2.72
	6	Check	Not treated			27	47	88	92	94	--------	15.6	91	8.57	--------	--------

†Based on original air dry weight of seeds.
*Based on oven dry weight of seeds.

to withstand the temperature of 95° for 12 hours is found in the amount of water contained by the seed when this is expressed as a ratio or as a percentage of the oven dry weight of the seeds. The oven dry weight of the seeds furnishes a nonvariable base for computation of the ratios or percentages expressing the moisture content. Without exception, when the moisture content after the preliminary drying was as great as 3.9 per cent of the oven dry weight, the viability of the seeds was seriously impaired by heating at 95° for 12 hours.

When the moisture content after the preliminary drying was not greater than 3.62 per cent of the dry weight of the seeds, no serious loss of viability resulted from the subsequent treatment at 95°. Furthermore, when the water content after drying was not greater than 3.19 per cent of their dry weight, the seeds heated at 95° for 12 hours germinated more promptly than the untreated seeds used for a check. The statement that, when the moisture content of cotton seed is not greater than 3.19 per cent of their dry weight, the seed may be safely subjected to a temperature of 95° for 12 hours without consequent loss of vaibility may then be considered to apply to any lot of normal cotton seed.

In the course of these studies certain other experiments were performed which further show the relation of time and temperature as factors influencing the water content of seeds during desiccation. In one of these experiments, cotton seeds were desiccated in a gas oven at a temperature of about 45° C., and at intervals of 24 hours the seeds were cooled in a desiccator, carefully weighed in order to determine loss of water and returned again to the oven. At the end of 144 hours, the temperature was raised to 60° C. and weighings were again made at the end of 24 and 48 hours. In another test seeds being dried at 60° C. in an electric oven were weighed at frequent intervals until their weight became practically constant. The temperature was then raised to 70° C. and the seeds were weighed after periods of 24, 48, 72, and 96 hours, and finally heated at 103° C. in order to determine their total water content. The results of these tests were presented in Table IV.

In experiment 7 the water content of the seeds rapidly diminished during the first 24 hour period of drying and continued to decrease markedly, but less rapidly, during the second and third periods. By the end of 72 hours, the weight of the seeds had became practically constant, only insignificant amounts of water, cumulatively less than 0.5 per cent, being lost during the three succeeding 24-hour intervals. When at the end of 144 hours the temperature was raised to 60° C., a marked reduction of water content of the seeds again occurred by the end of 24 hours. Little water was lost during the second 24-hour period of heating at 60°. By this time, the weight of the seeds and their water content had become nearly constant for the temperature of 60° C.

TABLE IV

SHOWING THE RELATION BETWEEN TIME AND TEMPERATURE AND LOSS OF WATER BY GIVEN LOTS OF SEED IN DRY AIR.

Exp. No.	Preliminary Desiccation		Subsequent Desiccation		Weight (Water) Lost		Increase in Per Cent of Water Lost
	Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Grams	Per Cent of Wet Weight	
7*	45	24	—	—	0.211	4.16	—
	45	48	—	—	0.242	4.77	.61
	45	72	—	—	0.270	5.33	.56
	45	96	—	—	0.275	5.42	.09
	45	120	—	—	0.279	5.50	.08
	45	144	—	—	0.289	5.69	.19
	45	144	60	24	0.360	7.10	1.41
	45	144	60	48	0.374	7.38	.28
8†	60	14	—	—	1.133	9.91	—
	60	23	—	—	1.185	10.37	.46
	60	40	—	—	1.216	10.64	.27
	60	62	—	—	1.234	10.80	.16
	60	86	—	—	1.237	10.82	.02
	60	86	70	24	1.292	11.31	.49
	60	86	70	48	1.322	11.57	.26
	60	86	70	72	1.329	11.63	.06
	60	86	70	96	1.32	—	—

*50 seeds, water content 10.57 per cent of wet weight, heated in gas oven.
†100 seeds, water content 13.20 per cent of wet weight, heated in electric oven.

In Experiment 8, in which seeds were heated directly at 60° C., weight and water content of the seeds rapidly decreased during the first 14 hours and continued to decrease, although at a diminishing rate, until it became constant at the end of 62 hours. When the temperature was raised to 70°, a marked decrease of weight again occurred during the succeeding period of 24 hours, and the water content again became practically constant by the end of 48 hours.

The results of these experiments are graphically illustrated in Fig. 1. The curves show the relation of time to loss of water from seeds which were being subjected to two different desiccatory dry heat treatments. Both curves rise rapidly at first and then flatten out indicating that longer desiccation at that particular temperature does not cause further dehydration. Later, the increase in the desiccatory temperature of the treatments is marked by an abrupt rise in each curve, and this is followed again by a flattening of the curves as desiccation once more approaches its maximum for the particular temperatures being used. If the temperature is too low, the time of desiccation may be prolonged indefinitely without attaining the degree of desiccation requisite to enable cotton seeds to withstand without loss of viability the higher temperatures which must be used to free it from anthracnose. On the other hand, when an adequate temperature is used, a more or less definite minimum of time is necessary in order to accomplish a degree of desiccation sufficiently pronounced to protect the seeds.

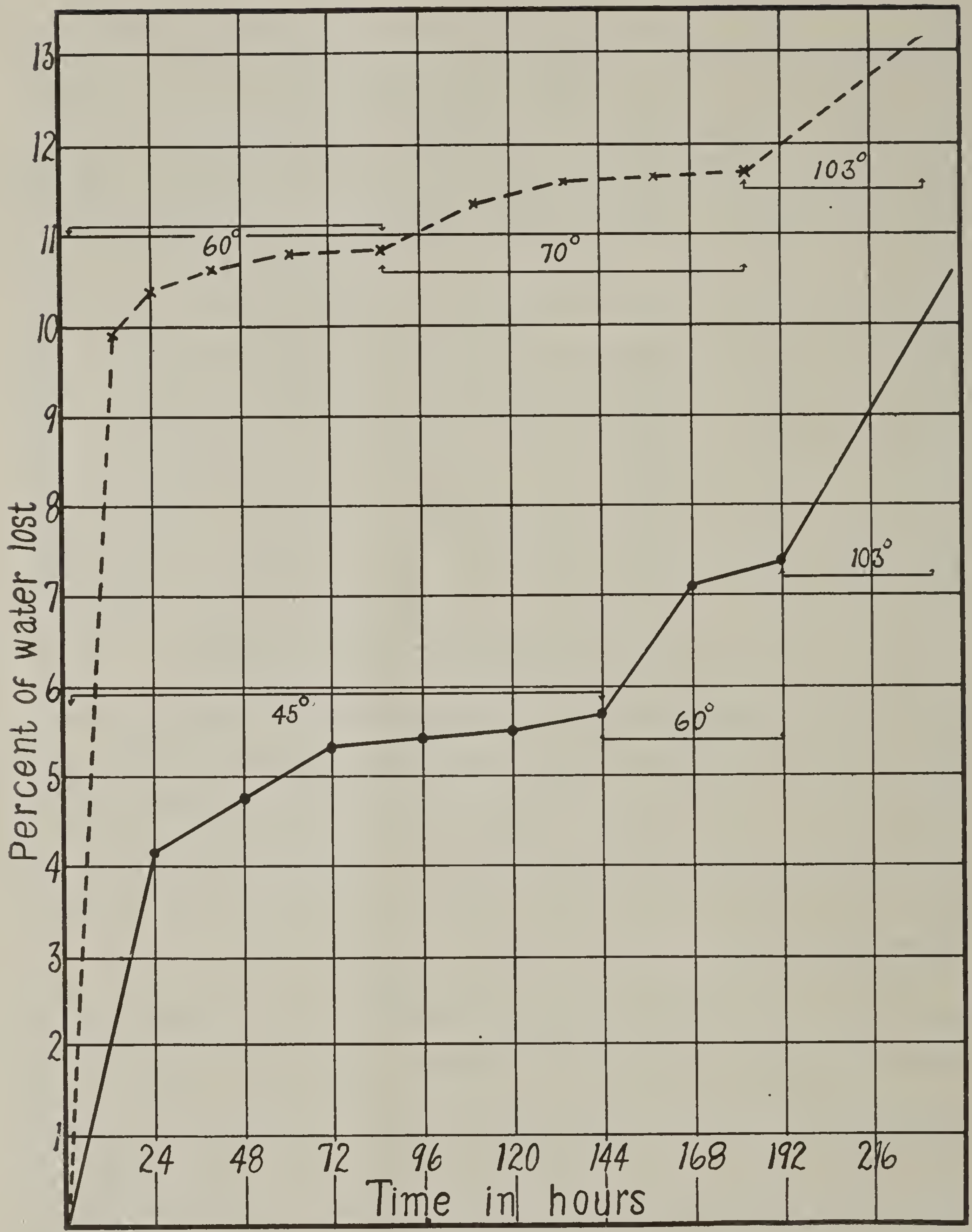

FIG. I

FIG. I. Curves which are based on the data presented in table IV and which show the relation between temperature and time of desiccation to loss of water by two lots of seed during desiccation. The curves rise rapidly at first but, after a more or less definite period of heating at a given temperature, each curve assumes a horizontal posiiton denoting equilibrium between the drying power of the air and the forces by means of which the seed retains water. When the temperature of drying is increased, each curve rises abruptly again and, after a time, assumes a horizontal course once more, indicating another point of equilibrium. Unless the temperature of drying is sufficiently high, seeds cannot be reduced to the state of desiccation which will enable them to withstand the high temperatures required to kill the seed-borne anthracnose fungus although the period of heating be greatly prolonged.

The lot of seeds represented by the lower curve was heated in a gas oven, and those represented by the upper curve, in an electric oven.

TABLE V

Accelerated Germination of Cotton Seed Following the Treatment to Destroy Anthracnose. 100 Seeds in each Lot.

Exp. No.	Lot No.	Preliminary Drying		Heating		Seed Coats	Per Cent of Germination at end of Time Indicated					Average Length of Radicle in mm.		Number of Seedlings Measured	
		Temp. Deg. C.	Time Hours	Temp. Deg C.	Time Hours		26 Hours	45 Hours	57 Hours	76 Hours	91 Hours	76 Hours	91 Hours		
1	1	60	18	95	12	Removed	62	88	88	93	-----	26.3	-----	54	-----
	2	Not Dried		Not Heated	-----	Removed	91	99	99	-----	-----	38.3	-----	62	-----
	3	60	18	95	12	Intact	1	23	87	93	95	-----	24.3	79	-----
	4	Not Dried	-----	Not Heated	-----	Intact	0	18	65	87	91	-----	21.2	80	-----
2	1	60–65	22	95–100	12	Removed	24 hrs. 38	47 hrs. 96	64 hrs. -----	112 hrs. -----	-----	72 hrs. 17.9	112 hrs. 32.5	88	-----
	2	Not Dried	-----	Not Heated	-----	Removed	75	100	-----	-----	-----	23.2	36.0	97	-----
	3	60–65	22	95–100	12	Intact	0	31	89	93	-----	-----	23.2	78	-----
	4	Not Dried	-----	Not Heated	-----	Intact	0	18	85	96	-----	-----	24.8	88	-----
3	1	60–65	22	95–100	12	Intact	46 hrs. 57	56 hrs. 82	78 hrs. 86	168 hrs. 86	-----	-----	-----	-----	-----
	2	Not Dried	-----	Not Heated	-----	Intact	40	80	90	91	-----	-----	-----	-----	-----
4*	1	60–65	22	95–100	12	Intact	46 hrs. 43	52 hrs. 69	71 hrs. 93	-----	-----	-----	-----	-----	-----
	2	Not Dried	-----	Not Heated	-----	Intact	42	68	92	-----	-----	-----	-----	-----	-----

*This Experiment differs from Exp. 9 only in that the blotters were permitted to become somewhat dryer before putting seeds to germinate.

ROLE OF THE SEED COATS

When Lipscomb and Corley[43] heated cotton seed in a vacuum or in an inert gas like nitrogen at 100° for 26 hours, they obtained a higher percentage of germination in treated than in untreated seed. Other workers as Haberlandt,[29] Wiesner[62] Velten,[60] Honing,[35] Davis,[12] Harrington[30] and others found that certain heat treatments accelerated germination or increased the total percentage of germination or both. In a number of the germination tests conducted by the writer, cotton seed which had been given certain treatments with dry heat appeared to germinate more rapidly than untreated seed. In order to determine whether this increase in rate of germination is due to some wholesome effect of the treatment on the embryo or whether it is due to some favorable change in the seed coat, certain experiments were planned. The results of these tests are set forth in Table V.

In experiment 1, four lots each of 100 seeds of the 1923 crop were used. The seed were carefully selected in order to have the different lots as uniform as possible. Lots 1 and 3 were dried at 60° for 18 hours, then heated at 95° for 12 hours. Lots 2 and 4, which were neither dried nor heated in any way, were used as checks on lots 1 and 3. The seed coats were removed from lots 1 and 2 and the embryos were put to germinate while the seeds of lots 3 and 4 were put to germinate with testas intact. The seeds were germinated between sterile blotters in pans holding 50 seeds each. Since each lot was germinated in two pans the method involved a check on such irregularities of germination that might obtain between one pan and another. Only the combined germination in the two pans representing each lot is given in the table. The number of seeds which had germinated was determined at the end of 25, 45, 57, 76 and 91 hours, and the average length of the radicles (hypocotyl and tap root) was determined at the end of the 72-hour period for the seedlings whose testas had been removed and at the end of the 91-hour period for the seedlings whose testas had been left intact. Only seedlings whose root and hypocotyl were healthy were measured, the number of measured seedlings being given in the table.

When the results of the tests with lots 1 and 2 are compared, it is seen that the embryos of treated seeds germinate more slowly than those of untreated seeds. At the end of 25 hours in the germinator only 62 treated embryos had germinated while 91 of the untreated embryos had begun to grow. At the end of the 45 hour period the germination was 88 and 99 respectively for the two lots and at the close of the 76 hour period the comparison was as 93 to 99. At the end of 76 hours, the radicles of the treated seeds were 26.3 mm. long while those of the untreated seed were 38.3 mm. in length. It is evident both from a comparison of the number of seeds which had germinated at a given time and a consideration of the average length of the hypocotyls that the treatment affected the embryos in such a way as to retard their rate of germination. The difference in the average length of the hypocotyls of the two lots does not appear to be due to more rapid growth of the untreated seedlings for measurements made on seedlings from treated and untreated embryos in lots 1 and 2 of Experiment 2 described below, at the ends of the 72 and 112 hour periods show slightly greater gain in length of the seedling from treated seed.

When a comparison is made of the results of the tests with lots 3 and 4 in which treated and untreated seed were germinated with testas intact, it is apparent that the rate of germination of the treated seed exceeded that of the untreated seed. At the end of the 45-hour period, 23 of the treated compared with 18 of the untreated seeds had germinated, and 12 hours later the relation was at 87 to 65. At the expiration of 91 hours, the germination of lot 3 still exceeded that of lot 4 slightly both in regard to number of seeds germinated and to the average length of the radicles of the seedlings.

In Experiments 2 and 3 the seed used had been treated in the seed-treating machine described in another section of this paper. Here, again, the rate of germination of naked embryos of treated seed was retarded in comparison with that of decorticated untreated seed; and, when seed were put to germinate with testas intact, the treated seed germinated more quickly than the untreated. Experiment 4 was exactly like Experiment 3 except that the sterile blotters were permitted to become slightly dry by standing in the laboratory for a time before the seeds were put to germinate. Under this condition of less rapidly available moisture the number of treated seeds which germinated exceeded the number of untreated so slightly as to be negligible yet the retardation of germination due to the effect of the treatment on the embryos was completely counterbalanced by the increased permeability of the testas of the treated seeds.

The removal of the seed coat permitted much quicker germination of both treated and untreated seed than when seed coats were intact, but the retarded rate of germination of the treated embryos was completely masked and even overcome by the restriction to germination imposed by the intact seed coat of untreated seeds. It is apparent that while the treatment affected the embryo in such a way as to prolong the period required for germination to begin it also produced a profound change in the testa increasing its permeability and more than compensating for the less prompt start of the treated embryos into activity.

The exact nature of this increase in impermeability of the testas of treated seeds has not been determined. It may be that fissures form in the seed coat as the result of heating and thus a more ready access of water to the embryo is provided as suggested by Detmer.[15] Denny[14] found that lipoids, tannins, and pectic substances were factors in determining the permeability of the coats of certain seeds. He found that the permeability of the seed coat of *Arachis hypogea* could be increased 28 to 50 per cent by heating the seeds in dry air at the temperature of boiling water. A much greater increase of permeability occurred when the seed coats were extracted for five minutes in boiling water.

The experiments described above may be summarized in the following words: The effect of desiccating cotton seed at relatively low temperatures varying from 40 to 60° C. for various periods of time has been studied as a means of conditioning the seed to endure without injury, temperatures of 80 to 95°, which are ordinarily harmful but are necessary to kill the anthracnose fungus. The loss of moisture by the seeds has been determined for the different stages of the treatment. The resistance of the treated seeds increased with increase in temperature and time of desiccation and is correlated with and dependent upon the loss of water during the period of desiccation. The reduction of the water content of seeds to 3.19 per cent of their dry weight fully prepares them to survive the temperature of 95° for 12 hours, such seeds germinating as completely and somewhat more promptly than untreated seeds. The acceleration in the rate of germination resulting from the treatment of properly desiccated seeds at 95° may be attributed to the effect of the treatment on the seed coats. When the preliminary desiccation has not been sufficiently great to fully protect the seeds from the injurious effects of the higher temperatures used in the final period of heating, the seeds show retardation in the rate of growth and more or less marked reduction in the total germination. Less severe injury may manifiest itself only as an increase in the time required for germination. This finding agrees with that of Nobbe,[14] Just,[39] Saillard,[47] Haberlandt[29] Höhnel,[34] Dixon,[16], [17] Störmer,[55], [56] Tapke,[57] and Crocker and Groves,[10] who found that temperatures near the maximum increase the time needed for germination.

EFFECT OF STORING DISEASED COTTON SEED OVER DESICCATING CHEMICALS

When it was learned in the early part of these studies with diseased cotton seed that the percentage of seeds carrying viable elements of the anthracnose fungus can be greatly reduced by use of dry heat, the question was raised whether the death of the anthracnose fungus is a result of the excessive loss of water while the seeds are being treated or is due primarily to a direct effect of heat. Is the withdrawal of water during the treatment sufficiently great in itself to produce fatal disruption of vital organization in the fungus protoplasm or is this permanent loss of vitality to be attributed to a direct, perhaps coagulating, effect of the heat on the living substances of the fungus? In order to arrive at an answer to this question, certain experiments were performed to determine the effect of removal of water from the diseased seed by desiccation over chemicals. Diseased seed of the 1919, 1920 and 1921 crops were stored over concentrated H_2SO_4 and dry CaO_2 in desiccators. The lids of the dishes were sealed with petroleum jelly and clamped down to prevent entrance of air from the outside. The dishes were kept at room temperature on a shelf of the general laboratory. Seeds were taken from these dishes from time to time and tested in the usual way for germination and anthracnose. In order to keep record of the degree of desiccation attained by the seeds, a small quantity of seed was sewed in a small cheese-cloth bag and stored in each desiccator. The weight of the bag and of the seed enclosed by it were determined at the beginning of the experiment. Each time seeds were removed from the desiccators for testing, the bag and seeds were again weighed and the amount of water lost by the seeds was recorded.

Table VI gives the data pertaining to these tests. In Experiment 1 seed of the 1919 crop was stored over concentrated H_2SO_4 in a desiccator dish. The seed were placed in the desiccator on July 21, 1920, approximately 9½ months after the seed had been harvested. Lots 1 and 2 were removed 58 and 139 days later respectively and although they had lost 10.2 per cen tand 10.8 per cent of their initial weight as water they still contained a high percentage of seeds with viable anthracnose. Lot 3 was removed from the desiccator after 210 days and at that time 20 per cent of the 50 seeds which were put to germinate or 28 per cent of the 35 seeds which did germinate still contained viable elements of the anthracnose fungus, while only 6 per cent of the seedlings of the check developed anthracnose. At the end of 269 days, when lot 4 was removed, the anthracnose had very largely disappeared from the treated seed, but even then, more than twice as many seedlings in the treated lot became diseased as in the untreated. The seed was by this time 18½ months old and had lost 11 per cent of their initial weight as water. Dry weight determinations made in the usual way on untreated seed gave an average of 10.88 per cent and indicate that 11 per cent represents the total initial water content of the seeds. This is a far more complete desiccation than is brought about by the dry heat treatments effective for freeing seed from anthracnose.

In Experiments 3 and 4, seeds of the 1920 crop were put to desiccate over con. H_2SO_4 and CaO_2. The experiments were begun on July 12, 1921, when the seeds were approximately 9½ months old and the first lot was removed from each desiccator 71 days later. When untreated seed were tested on April 6, 1921, approximately 97 days before they were put to desiccate, seedlings from 52 per cent of the seeds developed anthracnose. By July 30, 1921, 18 days after the experiments were started, the percentage of diseased seedlings in undesiccated seed had dropped to 34, and by August 15, 1921, this percentage had fallen to 10. At the time the first lot was removed from the two desiccators, namely, September 21, 1921, the anthracnose had completely lost its viability in the untreated seeds while it was still alive and capable of growth in 36 per cent and 26 per cent of desiccated seeds in the two experiments. The percentage of anthracnose found in untreated seed on July 30 was maintained till September 21 in desiccated seed, whereas in untreated seed it had completely died out by the latter date. The total water content of the seeds as indicated by a dry-weight determination was 9.6 per cent of their initial weight. The seeds of lot 1, Experiment 3, lost 8.7 per cent of their weight or 90.6 per cent of their total water content during the 71 days they were in the desiccator. Thus it appears from Experiment 3 that the vitality of the anthracnose fungus was prolonged approximately 53 days by removal of the greater part of the water from the seeds.

In Experiment 5, seeds of the 1921 crop were put over con. H_2SO_4 in a desiccator on January 19, 1922, at which time they were 3½ months old. Lot 1 was removed at the end of 196 days and at that time the percentage of diseased seedlings was slightly greater in the treated lot than in the untreated check. When lot 2 was removed, 28 per cent of the 50 seeds put to germinate or 46 per cent of the 35 seeds which did germinate by the end of 17 days in the desiccated lot were found to be carrying living elements of the anthracnose fungus, while at the same time the fungus was still

TABLE

PER CENT OF ANTHRACNOSE AND GERMINATION OF DISEASED COTTON SEED STORED

Exp. No.	Lot No.	Days in Des-iccator	Per Cent Water Lost	Days in Germinator Per Cent Anthracnose		Days in Per Cent				
				Treated	Check	Treated				
1	1	58	10.2	9 34%	8 34%	2 46%	3 54%	4 62%	5 62%	
	2	139	10.8	39 54%	--------	2 8%	4 50%	----------	8 64%	----------
	3	210	10.8	17 † 20%(= 28%)	17 6%	6 64%	12 68%	17 70%	27 * 70%	----------
	4	269	11.0	14 6% (= 9.6%)	14 4%	9 62%	14 62%	22 60%	41 ‡ 74%	----------
	5	356	11.0	--------------	--------	5 64%	6 66%	11 70%	24 76%	70 86%
2	1	60	7.4	27 2%	27 6%	6 64%	12 76%	17 88%	27 * 88%	----------
	2	116	8.3	22 0	22 4%	9 62%	14 64%	22 64%	41 ‡ 70%	----------
	3	225	8.8	--------------	--------	6 60%	11 74%	24 78%	70 86%	139‡ 90%
3	1	71	8.7	26 36%	26 0	7 90%	12 92%	26 * 92%	----------	----------
	2	387	9.4	14 0	14 0	9 86%	14‡ 86%	----------	----------	----------
4	1	71	8.9	26 26%	26 0	7 86%	12 86%	26 * 90%	----------	----------
	2	409	9.6	14 0	14 0	7 90%	14 ‡ 98%	----------	----------	----------
5	1	196	7.8	35 52% (=74%)	35 60%	9 48%	14 52%	19 58%	28 66%	35* 70%
	2	424	------	17 28% (=46%)	17 20%	17 60%	---------- ----------	---------- ----------	---------- ----------	---------- ----------
	3	540	7.8	42 16% (= 27%)	42 2%	3 16%	4 30%	10 44%	13 44%	19 46%

*See text for further germination.

†Anthracnose per centage calculated on basis of the number of seeds which were put to germinate and in parenthesis on basis of number which actually germinated.

‡All remaining seeds dead.

VI

FOR VARIOUS PERIODS OVER DESICCATING CHEMICALS. 50 SEEDS IN EACH LOT.

Germinator						Year When Harvested	Age When Put to Desiccate mo.	Age of Seed When Tested mo.	Desiccating Agent
Germination									
					Check				
7 62%	----------	9 62%	17 * 66%	----------	----------	1919	9½	11½	Con H_2SO_4
20 72%	----------	31 78%	39 80%	48 * 80%	----------	1919	----------	14	Con H_2SO_4
----------	----------	----------	----------	----------	6 98%	1919	----------	16½	Con H_2SO_4
----------	----------	----------	----------	----------	9 96%	1919	----------	18½	Con H_2SO_4
139 ‡ 90%	----------	----------	----------	----------	6 82%	1919	----------	21½	Con H_2SO_4
----------	----------	----------	----------	----------	6 98%	1919	14½	16½	CaO_2
----------	----------	----------	----------	----------	9 96%	1919	----------	18½	CaO_2
----------	----------	----------	----------	----------	6 82%	1919	----------	21½	CaO_2
----------	----------	----------	----------	----------	7 98%	1920	9½	11½	H_2SO_4
----------	----------	----------	----------	----------	9 94%	1920	----------	22	H_2SO_4
----------	----------	----------	----------	----------	7 98%	1920	9½	11½	CaO_2
----------	----------	----------	----------	----------	7 94%	1920	----------	22½	CaO_2
----------	----------	----------	----------	----------	9 100%	1921	3½	10	H_2SO_4
----------	----------	----------	----------	----------	17 94%	1921	----------	17½	H_2SO_4
42 58%	102 * 64%	----------	----------	----------	4 96%	1921	----------	21½	H_2SO_4

viable in only 20 per cent of the seeds of the untreated check. When lot 3 was removed, 16 per cent of the 50 seeds put to germinate or 27 per cent of the 32 seedlings which grew became diseased, while only 2 per cent of the untreated check developed anthracnose. Thus, it appears that in 26 per cent (46%-20%) of the desiccated seeds which germinated in lot 2, the vitality of the anthracnose fungus was prolonged to 17½ months by storage in the desiccator, whereas, under ordinary conditions of laboratory storage, the fungus in these seeds would have perished by this time. Likewise, as indicated by lot 3, the anthracnose fungus was preserved in a viable condition to 21½ months in 25 per cent (27%-2%) of the treated seeds.

The results of Experiment 2 are not in complete harmony with those of the other tests, although the seed were from the same bag as those used in Experiment 1. In tests made at the end of 60 and 116 days fewer seedlings became diseased in treated than in untreated seed. This anomalous outcome is probably dependent on the fact of the more advanced age of the seed when put to desiccate. In Experiment 1, the seed were put to desiccate on July 21, 1920, when they were 9½ months old, while in Experiment 2, they were put in the desiccator on December 18, 1920, at which time they were 14½ months old. When untreated seed were tested on July 1, 1920, 66 per cent of the seedlings became diseased; on Sept. 17, 1920, 34 per cent and on January 18, 1921, just one month after Experiment 2 was begun, only 6 per cent of the seedlings in one lot and none in another became diseased. Thus it is seen that when Experiment 1 was started a large percentage of the seeds were carrying the anthracnose fungus in a viable condition while by the time Experiment 2 was begun the seed had passed through the summer months and the anthracnose fungus in most of the seeds had died as a result of ageing. Until two weeks before Experiment 1 was started, these seed were stored in an out-building; after this time they were kept in the laboratory. During the period of storage in the laboratory before Experiment 2 was begun the water content of the seeds decreased from approximately 11 per cent to approximately 9 per cent. In Experiments 3 and 4, it is shown that the desiccating agents removed all or nearly all the free water from the seeds, and it is assumed that this is likewise the case in Experiments 1 and 2.

From the experiments described above, it is seen that the vitality of the seed-borne elements of the cotton anthracnose fungus is prolonged and not shortened by storing diseased seed at room temperature over desiccating chemicals such as con H_2SO_4 and dry CaO_2. This increase in the length of life of the fungus appears to be conditioned by a pronounced loss of water from the seeds and is probably due primarily to a decided decrease of the already low respiratory activity of the fungus and seed. When diseased seed are treated with dry heat, it is not the desiccation which destroys the life of the anthracnose fungus, but the direct, presumably coagulating effect of the heat on the fungus protoplasm, which appears to be less resistant to heat than the protoplasm of the seed itself. The reduction of the water content of seeds which are being treated is sought only as a means of conditioning the seed to withstand the ordinarily injurious effects of the high temperature necessary to kill the invading fungus.

INDUCED SECONDARY DORMANCY OF COTTONSEEDS

In addition to the increase in length of life of the anthracnose fungus as the result of storing diseased cotton seed in desiccators kept at room temperature, an induced secondary dormancy of more or less prolonged duration was observed to occur in a considerable proportion of the stored seeds. The results of these germination tests are given in part of Table VI.

In Experiment 1, lot 1, 46 per cent of the seeds germinated in 2 days; 54 per cent in 3 days; 62 per cent in 4 days and 62 per cent in 9 days. The seeds were not examined again until the 17th day at which time 66 per cent had germinated. 17 seeds had not germinated by the end of 17 days. Seven of this number were covered with fungus, the seed coats being soft and the embroys dead and partially decayed. Eight of the 17 seeds had seed coats which were extremely dry within and much harder than those of untreated seeds. These seeds had not swollen and had imbibed no water as indicated by the dry condition of the surface of the embryo. When the hard seed coats were removed and the dry embryos were placed on moist blotting paper, 7 of the 8 seeds germinated and produced hypocotyls varying from 1 to 15 mm. within 24 hours, and all germinated in 3 days. The remaining two of the 17 seeds had already imbibed considerable water, the coats being soft and the embryos moist when the seeds were removed from the germinator on the 17th day. These two seeds also germinated promptly when their testas were removed. Obviously, they had already overcome the dormancy imposed by their dry seed coats and were at the point of germination when the seed coats were removed.

In lot 2, which lost 10.8 per cent of its weight while it was in the desiccator, 8 per cent of the seeds germinated in 2 days and 50 per cent in 4 days. Thereafter the percentage of germination increased very slowly until it reached 80 per cent on the 39th day. No further increase had occurred by the 48th day and the seeds were then removed from the test tubes. Six of the 10 which had not germinated were dead and covered with fungous growth while 4 possessed seed coats which were hard and dry within and had apparently absorbed no water. The testas were removed and the seeds were put on moist blotting paper whereupon all germinated and produced radicles about 3 cm. in length within 3 days. In lot 3, germination increased from 64 per cent on the 6th day of 70 per cent on the 17th day while 98 per cent of the seeds of the untreated check germinated in 6 days By the 27th day, 15 of the desiccated seeds had failed to germinate, 7 having hard and dry testas and 8 being dead. The 7 seeds with hard testas germinated at once when seed coats were removed. In lot 4, the germination increased from 62 per cent on the 9th day to 74 per cent on the 41st day at which time all the remaining ungerminated seeds were dead, while 96 per cent of the untreated check germinated in 9 days. In lot 5, which had been in the desiccator 356 days and had lost 11 per cent of its weight as water, the percentage of germination increased by slow increments from 64 per cent on the 5th day to 90 per cent on the 139th day, by which time all remaining seeds had died. The untreated check gave 82 per cent of germination in 6 days.

In Experiment 2, in which CaO_2 is used as the desiccating agent, germination was prolonged by reason of the induced condition of secondary dormacy. At the end of 27 days, 6 seeds of lot 1 had not germinated, 3 of the 6 being dead and 3 having hard, dry seed coats. The embryos of the 3 seeds with hard coats imbibed water and germinated promptly after the seed coats were removed. In lot 3, germination was greatly prolonged increasing slowly from 60 per cent on the 6th day to 90 per cent on the 139th day at which time all remaining seeds were dead.

In Experiment 3, seed of the 1920 crop were stored over concentrated H_2SO_4 and in Experiment 4 over dry CaO_2. In these two experiments the seeds were highly desiccated as indicated by the percentages of water lost, but germination was only slightly prolonged. In the case of lot 1 of each experiment, the germination was 92 and 90 per cent respectively in 26 days while the checks gave a germination of 98 per cent in 7 days. No note was made of the condition of the seeds which had not yet germinated at the end of 26 days. A portion of these might have germinated later if they had been kept for a longer time. In the case of lot 2 all seeds which had not germinated on the 14th day were dead.

In Experiment 5, seeds of the 1921 crop were stored over concentrated H_2SO_4. The germination of lot 1, removed from the desiccator at the end of 196 days, increased from 48 per cent on the 9th day to 70 per cent on the 35th day. At that time, 8 of the remaining 15 ungerminated seeds were dead and 7 were still alive. The untreated check yielded perfect germination by the 9th day. Only 60 per cent of the seeds of lot 2 germinated in 17 days while 94 per cent of the untreated seeds grew in the same time. Lot 3 was removed from the desiccator at the end of 540 days. The germination increased by small increments from 16 per cent on the 3d day to 64 per cent on the 102d day when the experiment was discontinued. 96 per cent of the untreated seeds germinated in 4 days. The seeds used as checks in all the above mentioned experiments were drawn originally from the same bag as the treated seeds and were handled in the same way except that they were kept in the original bag while the treated seeds were stored in the desiccators.

In 3 out of 5 experiments in which cotton seed were desiccated with chemicals, the period required for complete germination of the viable seeds was markedly prolonged in comparison with the time required for germination of the undesiccated seed. The treated seed are put into a condition of more or less complete secondary dormancy or restricted activity. This is due primarily if not wholly to the desiccation of the testas of such extreme nature that they become non-wetable with water at the germination temperature of about 25° C. In the presence of abundant moisture the desiccated condition of the seed coat is reversed slowly at a rate which differs with individual seeds and which is probably dependent upon the degree of desiccation of individual seeds. Consequently the germination of such seeds is slow and very irregular.

The phenomenon of delayed germination of seeds is exhibited by a very considerable number of species. It is due to conditions which fall into two general categories, namely, those resident in the embryos and those due ito restrictions imposed by the seed coats.[7] Thus the embryos of hawthorn[8] and juniper[46] are dormant and must pass through a period of after-ripening, the time of which may be shortened by appropriate treatments before germination can take place.[8], [13], [19]. On the other hand, delayed germination in Xanthium[8], [46] and *Avena fatua*[1] is due not to dormant embryos but primarily to seed coat structures preventing the entrance of oxygen; in *Axyris amaranthodides* and *Chenopodium ablum*[8] and *Lathyrus maritimus*,[52] to seed coats retarding or preventing the entrance of water; and in Iris,[8] *Alisma plantago*,[11] other water plants,[9] and *Diospyros virginia*,[5] to the great strength of the seed coat which mechanically prevents swelling of the embryo before the latter has imbibed sufficient water to permit germination. With Ilex[37] there obtains a combination of conditions falling in the two general categories mentioned above. The embryos are not dormant but very immature and require a long period of growth before germination occurs but even then the weak hypocotyl is unable to penetrate the woody pericarp until the latter has decayed sufficiently.

Seeds of certain plants which are capable of immediate germination may be thrown into a condition of secondary dormancy. Kidd[40], [41] subjected seeds of *Brassica alba* to high partial pressures of CO_2 and found that the treated seeds became dormant and remained in that condition indefinitely after removal from the inhibiting CO_2 pressures. The dormant condition could be broken only by redrying and rewetting or removal of the testas. Crocker[7] cities other instances in which secondary dormancy has been imposed on seeds by treatments which induced restrictive seed coat changes. However, in the cases of secondary dormancy induced in *Brassica alba* seeds by Kidd and in the cases cited by Crocker the dormant condition was induced while the seeds were in the moist condition favorable for germination. On the contrary, the secondary dormancy exhibited by the cotton seeds described above was induced by excessive drying in desiccators, and is more nearly comparable to the wide-spread hard shell condition characteristic of so many of the Leguminoseae but found in certain other families, among which are the Malvaceae, the family of which cotton is a member, Guppy.[28] The writer has found the hard-shell condition of cotton seeds occurring naturally in only one instance. Some of the seeds used by the writer from the 1924 crop had come to maturity during a season unfavorable for the best development of cotton seeds and among these one seed in a lot of 100 was occasionally found which did not germinate because of its hard shell condition. According to Toole and Drummond,[59] "If the seeds dry out until the moisture content is between five and six per cent, there is a marked development of 'hard seeds' which fail to take up water at all." A similar hardshell condition which retards or prevents germination of beans was described by Gloyer[23] in 1921, who ascribed the condition to extreme drying while stored in artifically heated rooms or when ripening occurs in dry, hot weather.

EFFECT OF STORING INFECTED COTTON SEED IN HYDROGEN AND IN CARBON DIOXIDE

A number of experiments have been made to determine the effect of an atmosphere of hydrogen and of carbon dioxide on the vitality of diseased cotton seed and on the logevity of the spores and mycelium of the anthracnose fungus carried by such seed. As in previous experiments, the seeds used were selected in order to remove all seeds which were, so far as could be judged from external appearances, incapable of germination. These selected seeds were stored in lots of several hundred in 750 cc. bottles provided with 2-holed rubber stoppers. The stoppers were vaselined, pressed tightly into the bottles and tied down with strong cord. The gases used were made to displace the air in the bottles containing the seeds by passing into them by way of 2-holed stoppers quantities of the desired gases equal to several times the volume of the bottles. When the air in the bottles had been displaced, the holes in the stoppers were closed by pushing tightly fitting vaselined glass rods into them. The bottles were were then kept on a laboratory shelf at room temperature. The bottles were recharged with appropriate gases each time samples of seeds were removed for germination tests.

The hydrogen used was produced by the action of zinc and sulphuric acid in a Kipp generator and was passed through wash bottles containing H_2SO_4 KOH solutions. In Experiment 1, the hydrogen was dried by passing it through a tube filled with CaO_2. The carbon dioxide used was produced by the interaction of H_2SO_4 and air slacked lime in a flask provided with suitable outlets and was used without further purification. In Experiments 1 and 2 the untreated seeds used for checks were kept in

TABLE VII

Per Cent of Anthracnose and Germination in Cotton Seeds Stored in Carbon Dioxide and in Hydrogen. 50 Seeds in Each Lot.

Exp. No.	Lot No.	Gas Used	Per Cent Germination		Per Cent Anthracnose		Age of Seed		Year Harvested
			Treated Seed	Untreated Seed	Treated Seed	Untreated Seed	When Stored	When Tested	
1	1	H_2	98	100	6	60	3½ mo.	10 mo.	1921
	2	H_2	90	100	8	32	3½	10½	1921
	3	H_2	88	94	2	20	3½	17½	1921
2	1	CO_2	96	100a	32	60a	3½	10 mo.	1921
	2	CO_2	96	100a	26	32a	3½	10½	1921
	3	CO_2	92	94a	2	20a	3½	17½	1921
3	1	H_2	94	98	58	90	6 mo.	10½ mo.	1922
	2	H_2	100	96	36	68	6	13	1922
4	1	CO_2	92	96b	50	68b	6	13 mo.	1922
5	1	H_2	92	98	28	28	5 mo.	6 mo.	1923
	2	H_2	98	98	26	26	5 mo.	9 mo.	1923
6	1	CO_2	96	98c	28	28c	5	6 mo.	1923
	2	CO_2	94	98c	40	26c	5	9	1923

aUntreated seeds same as for Exp. 1.
bUntreated seeds same as for Exp. 3.
cUntreated seeds same as for Exp. 5.

an open dish and in Experiments 3 to 6 they were kept in tightly stoppered bottles. The seeds were germinated in test tubes and at a temperature of about 25° C. Each lot tested in each experiment consisted of 50 seeds and the percentage of germination and the percentage of seeds which developed diseased seedlings was determined for each lot at intervals of 6 to 8 days, but only the total percentages obtained at the time the germination test was discarded are given here. The results of these experiments are presented in Table VII.

In two tests as high or higher germination was obtained with the seeds which had been kept in the single gases as with those stored in air. In all remaining tests the treated seeds gave slightly lower germination than the untreated seeds. The differences, however, are slight, and the tests show that cotton seed can suspend aerobic respiration and live in the absence of free oxygen for at least fourteen months.

This situation is not unusual among seeds. Houdas[66] found no reduction in germination of seeds of *Gerbera Jamesoni* after ten years in atmospheres of hydrogen and carbon dioxide. Becquerel[4] and others have shown that seeds may live several months or years in total absence of air, and Laurent[42] and Guillaumin[27] found that seeds of a number of common plants, particularly oleagenous kinds, stored in high vacuum for periods of 5 and 7⅓ years were conserved better than when stored in air. Babcock[2] found that air dired corn containing 10 per cent of moisture can be stored in CO_2 for a year without harm but under similar storage corn containing 20 per cent of water loses its power to germinate in less than two months. Edquist[20] found that grain can be stored in an atmosphere of pure nitrogen and carbon dioxide without injury to the grain, but the eggs of weevils and other insects are prevented from developing.

In regard to the effect of the treatment on the vitality of the anthracnose fungus, the results of the tests with seed produced in different years are highly discordant. In Experiment 1, in which seed grown in 1921 were stored in hydrogen gas the percentage of anthracnose, computed on the basis of the number of seeds put to germinate, at the end of seven months of storage in the gas was found to be 32 in the untreated and in 8 in the treated seeds and at the end of 14 months of storage the percentage was 20 for the untreated and 2 for the treated seed. In this experiment, a very pronounced reduction but incomplete suppression of disease appears to have resulted from storage of the seeds in hydrogen. In Experiment 2, which differed for Experiment 1 in the single condition that CO_2 was used instead of H_2, 32 per cent of anthracnose was found in untreated and 26 per cent in treated seed after 7 months of storage and 20 per cent was found in the untreated as compared with 2 per cent in the treated after 14 months of storage in the gas. The reductions are much less pronounced than in Experiment 1 except for the longest period of storage in the two gases. In Experiments 3 and 4 some reduction in the amount of disease occurred in the seeds stored in H_2 and CO_2 but the amount is much less than in Experiments 1 and 2. Again, the action of the hydrogen appears to be more efficacious than the CO_2 in reducing disease. In Experiments 5 and 6 in which seed of the 1923 crop were treated, no reduction in viability of the anthracnose had occurred after 4 months storage in either hydrogen or carbon dioxide.

A number of investigators have shown that the permeability of cellular membranes to gases depends upon their state of humidity. Becquerel[4] working with lupin, pea, and bean (Faba) found that their seed coats in an air dry condition are impermeable in all their parts to dry air, but when the same seeds are exposed to an atmosphere saturated with water vapor, the integuments imbibe sufficient moisture to permit the slow passage of gases. No one has studied the permeability of the testas of cotton seeds to hydrogen and carbon dioxide but the work of Becquerel and others indicates that the permeability decreases with increasing desiccation and may actually become nil at the degree of dryness attained when seeds are stored under laboratory conditions. While no moisture determinations were made on the seeds used in the writer's tests described above, it is known that the seeds in Experiments 1 and 2 in which the amount of anthracnose was markedly reduced in the treated lots, were stored in the gases very soon after their arrival at the laboratory. On the other hand, the seeds used in Experiments 3, 4, 5, and 6 in which less reduction of anthracnose occurred, remained for a considerable time in the dry air of the laboratory before the experiments were started and may have become, by reason of more thorough desiccation, less permeable to the gases in which they were kept.

The only conclusion that can safely be drawn from these experiments is that storage in CO_2 and H_2 cannot be relied upon to free cotton seeds from infection by the anthracnose fungus. This agrees with the finding of Barre and Ludwig,[3] who reported that storage of infected cotton in gases did not free the seed from the anthracnose disease.

GENERAL CONCLUSIONS

In order to enable cotton seeds to withstand, without loss of viability, the high temperatures to which they must be subjected in the final stages of the dry heat treatment for anthracnose, it is essential that their water content be reduced to a small fraction of that which is commonly found in seeds stored under ordinary conditions. This preliminary desiccation can be accomplished conveniently and most rapidly by drying the seeds at a temperature too low to harm them. The degree of dehydration required varies in direct manner with the temperature which is to be used in the final stages of the treatment and is fully attained by reducing the water content of seeds which are to be heated for 12 hours at 95° to a value which is equivalent to 3.2 per cent of their air dry weight. Both the temperature and the time of drying are factors of primary significance in this operation. If the temperature is too low, the time of drying can be extended indefinitely without accomplishing the desired degree of desiccation. The absorptive power of the dry air comes into equilibrium with the hydrophyllic properties of the colloids and other constituents of the living cells of the seed before the state of desiccation which will enable the seed to survive high temperatures has been reached. On the other hand, when a temperature which is sufficiently high is used, a more or less definite minimum of time is necessary to produce a degree of desiccation sufficiently pronounced to protect the seeds. A temperature of 45° C. cannot produce sufficient reduction in the water content of seeds to enable them to endure 95° for 12 hours, but 50° for 36 hours or 60° for 18 hours gives fairly adequate protection. As shown in Part I of these studies on cotton seed treatment, the same kind of time and temperature relation holds for the death of the anthracnose fungus carried on diseased seed. Heating at 45° for 168 hours does not completely free the seed from anthracnose but this result is fully accomplished by 12 hours of heating at 95°.

When seeds have been adequately dehydrated before the application of the high temperature in the second stage of the anthracnose treatment described in Part I of these studies, germination is somewhat more prompt than in untreated seeds. This acceleration of germination of treated seeds is not conditioned by any wholesome effect of the treatment on the embryos but is due entirely to favorable changes in the seed coat which facilitate the passage of water to the embryo; for, when naked embryos of comparable lots of treated and untreated seeds are compared with respect to promptness of germination, the embryos of untreated seeds are seen to germinate more promptly than those of treated seeds. When the seed coats are intact, the less rapid germination of the embryos of treated seeds is completely masked and more than compensated for by the favorable changes which were produced in the seed coats. The increased permeability of the coats of treated seed may be due to the formation of fissures or to the induction of chemical changes in the seed coat by the high temperatures used in the treatment. When the preliminary desiccation has not been sufficiently great to fully protect the seeds from the injurious effects of the higher temperatures used in the final stages of the treatment, the seeds show retardation in rate of growth and more or less marked reduction in total germination. Less severe injury may manifest itself only as an increase in the time required for germination.

Desiccation itself is not responsible for the death of the anthracnose fungus carried on diseased cotton seed; for, when infected seeds are thoroughly dehydrated at room temperature over chemicals, the fungus actually lives longer than in seeds containing a normal amount of water. This increase in length of the life of the fungus appears to be conditioned by the pronounced loss of water from the seeds and is probably due fundamentally to a decided decrease in the already low oxidative and respiratory activities of the fungus and seed. This condition of prolonged viability in the uniformly dry atmosphere of the desiccators is in harmony with the findings of Duvel,[18] who reported that the climate, particularly moisture and temperature of the place where seeds were kept, exerted a marked influence on their length of life, and of Tillotson,[58] who reported that seeds of coniferous trees were little if any affected by geographic location and were better preserved when kept in air-tight glass jars than when stored in bags or other open containers. Moreover, this condition of prolonged viability in the desiccators shows that the death of the anthracnose fungus when diseased seeds are treated with dry heat is not due to desiccation, but is to be attributed to a more direct effect of the heat, which may act by irreversibly coagulating the fungus protoplasm. That the death of the fungus is not due primarily to oxidative changes induced in the fungus protoplasm is indicated by the experiments of Lipscomb and Corley[13] who reported that the fungus is killed when dry diseased seed are heated at 100° in a vacuum or in an atmosphere of nitrogen. The reduction of the water content of seeds which are to be treated for anthracnose is sought only as a means of conditioning them to withstand the ordinarily injurious effects of the high temperatures necessary to kill the invading fungus.

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JULY, 1925 TECHNICAL BULLETIN No. 27

FOWL TYPHOID
and
FOWL CHOLERA

BY
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THE AGRICULTURAL EXPERIMENT STATION
OF THE
NORTH CAROLINA STATE COLLEGE OF AGRICULTURE
AND ENGINEERING

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STATE COLLEGE STATION
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Bulletins of the Station will be sent to citizens upon request

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INTRODUCTION

The problem of disease control has been a subject of research and study since the early ages. The advancement of civilization has seen monumental achievement in the way of prevention and cure. Man's energies have not been expended alone on his own creature, but has extended to all branches of livestock, plants and other animate objects that suffer from invasion of other biologic orders.

The poultry world, like other orders, suffers from devastating contagiums. The likelihood of the spread of disease among poultry is enhanced by their living conditions. Intensive raising and forced production has separated the domestic fowl so widely from its original primitive condition that its susceptibility to disease is greatly increased.

In North Carolina the poultry industry has experienced rapid growth in the past twenty years. The state presents unexcelled soil and climatic advantages for this branch of livestock production, and is within a reasonably short distance of excellent markets.

Salmon's original survey of diseases in poultry in 1880 showed eight of twenty-two counties in North Carolina reporting the loss of birds from cholera, Robeson County, in particular, reporting the loss of thousands of birds from cholera and sore head annually. It is evident from recent researches that a large amount of the disease reported as cholera was avian typhoid. Contagious disease outbreaks have kept pace with the growth of the industry, and there has been no organized effort to keep them within control. Such a condition is prone to open the industry to serious losses because of the contagious nature of most of the avian diseases of bacterial origin, and the fact that the soil occupied by diseased birds retains virulent germs for an undetermined length of time, making it hazardous to introduce new birds on such infected land.

The information of the average poultryman as to poultry diseases is meagre. Many times disease outbreaks are allowed to become well established before the seriousness of the condition is recognized, and then efforts at eradication of the disease are usually unscientific and ill handled, and heavy loss ensues.

A rapid correction of such conditions is impossible. It must be brought about by education through all possible agencies. Thorough training of County Agents and Agricultural High School teachers in the prevention and diagnosis of poultry diseases, and a coördination of the extension and experiment station activities with such agencies should be helpful in reducing the high mortality rate that is found in the poultry industry today. It is on the hopes of a brighter tomorrow that the efforts of today are founded.

FOWL TYPHOID AND FOWL CHOLERA

By
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and
R. S. DEARSTYNE, *Associate Research Professor*
Department of Poultry Science

THE PROBLEM

The contents of this bulletin is the results of research on Project 162, as outlined in Bulletin 247 of the Experiment Station of the North Carolina State College. The subject is that of investigation of Septicaemic Diseases of Poultry in North Carolina, a condition which has proven of great economic importance to the state. The problem involves the determination of the prevalence of avian typhoid and of avian cholera; the nature of these diseases; their importance from an economic standpoint, and possible methods of prevention and control.

METHODS OF APPROACH

The method of approach is the study of outbreaks of typhoid and cholera in the field as to causation, dissemination, and control, and to conduct studies of the bacteriology, sereology, and pathology of these diseases by inducing the diseases through artificial infection.

EXPERIMENTAL PROCEDURE

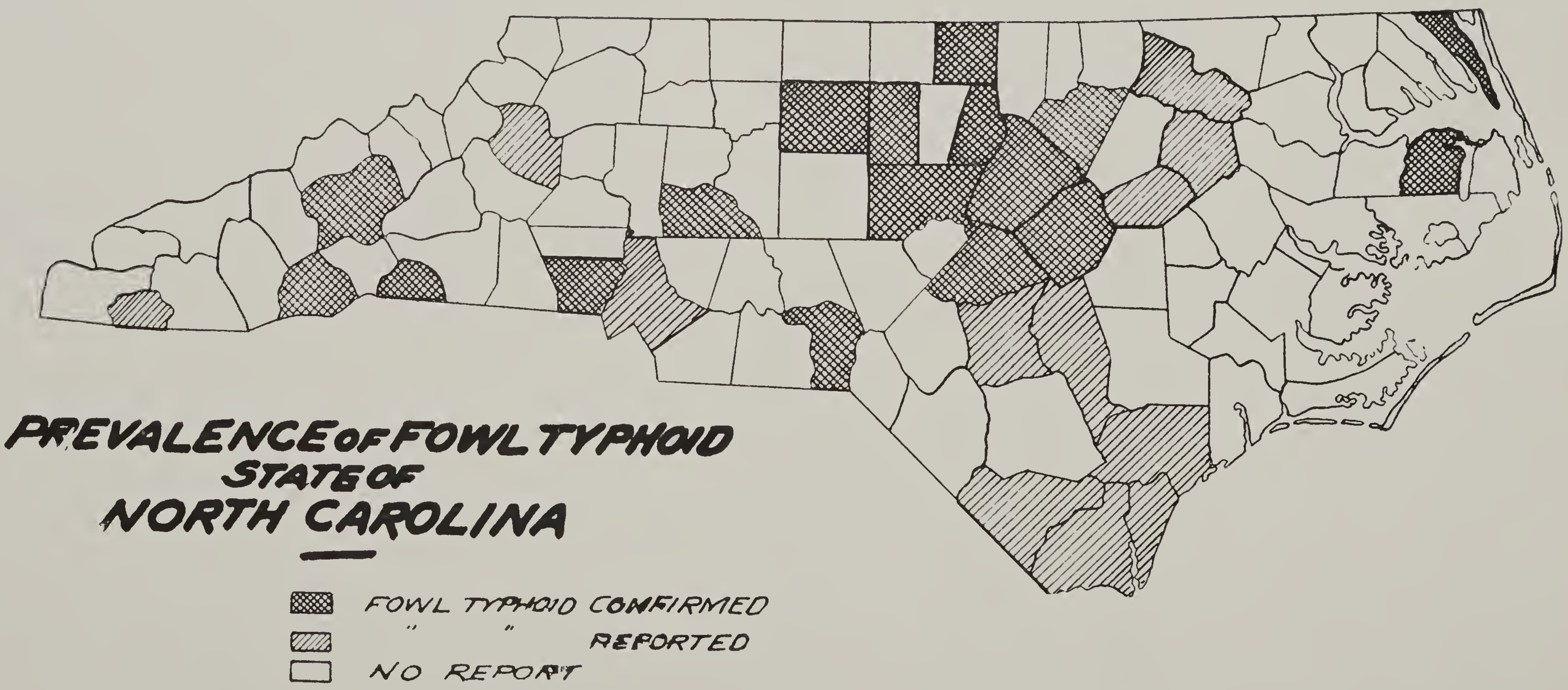
Experimental procedure included a study of the causative organisms of avian typhoid and avian cholera in accordance to Methods of Pure Culture Study as adopted by The Society of American Bacteriologists; field studies of symptomotology; mortality index; methods of dissemination, such as infected soil, mechanical conveyors, wild and domesticated birds and animal carriers; the possibility of transmission of the diseases through the eggs; clinical studies of artificial infection cases, including respiration, temperature, gross and microscopic pathology, histological changes, and the sereological reactions which accompany the disease.

Finally, a comparative study of the diseases of avian typhoid and avian cholera was made.

PREVALENCE OF THE DISEASE

It is highly probable that outbreaks of avian typhoid are to be found in all countries where poultry husbandry is practiced.

Reference to the Historical Resumé will show that as early as the development of bacteriological science would admit of the isolation and identification of disease-producing organisms, typhoid-like epizootics among poultry were studied, and causative organisms isolated. The historical record shows that the epizootics assumed large proportions, with thousands of birds succumbing annually to the disease. The original researches on poultry diseases were localized to southern and central Europe, this fact emphasizing the presence of the contagium in countries in that area, though it is probable that the condition existed in all countries.


PREVALENCE OF FOWL TYPHOID
STATE OF
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FOWL TYPHOID CONFIRMED
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FIG. 1.—PREVALENCE OF FOWL TYPHOID IN NORTH CAROLINA

In 1924, Truche and Staub (32) reports avian typhoid as being of common occurrence in France. This same report is made of Italy in the same year, by the Minister of the Interior (22). In 1922, teHennepe reports 73 outbreaks of Kleins Disease (avian typhoid) in The Netherlands.

Salmon (28) in 1880 made a general survey of poultry diseases in the United States, special stress being laid on "Cholera." Since the investigations of Salmon, such light has been thrown on Cholera-like diseases in poultry that it seems highly probable that a large percent of the "cholera" reported was avian typhoid. Of 27 states or territories reporting, 19 of them reported cholera outbreaks among chickens. Kaupp (13) in 1912, reported outbreaks of fowl typhoid among ducks, turkeys, and chickens in Colorado. In 1916, Taylor confirmed an outbreak of avian typhoid in California. Mulsow, in 1919, made a survey through experiment stations of the prevalence of the disease in the United States. California, Delaware, Iowa, Missouri, Pennsylvania, Washington, and Wyoming reporting rare outbreaks of the disease, while Connecticut, Massachusetts, and Rhode Island reported frequent outbreaks. Twelve states reported no studies made, and in thirteen no epidemics were observed. The work of Bushnell and Patton on the Use of Vaccines in Poultry Husbandry, in 1924, gives the impression that the disease is of frequent occurrence in Kansas, and the work of the present investigations of this station shows that the disease is prevalent under Southern conditions.

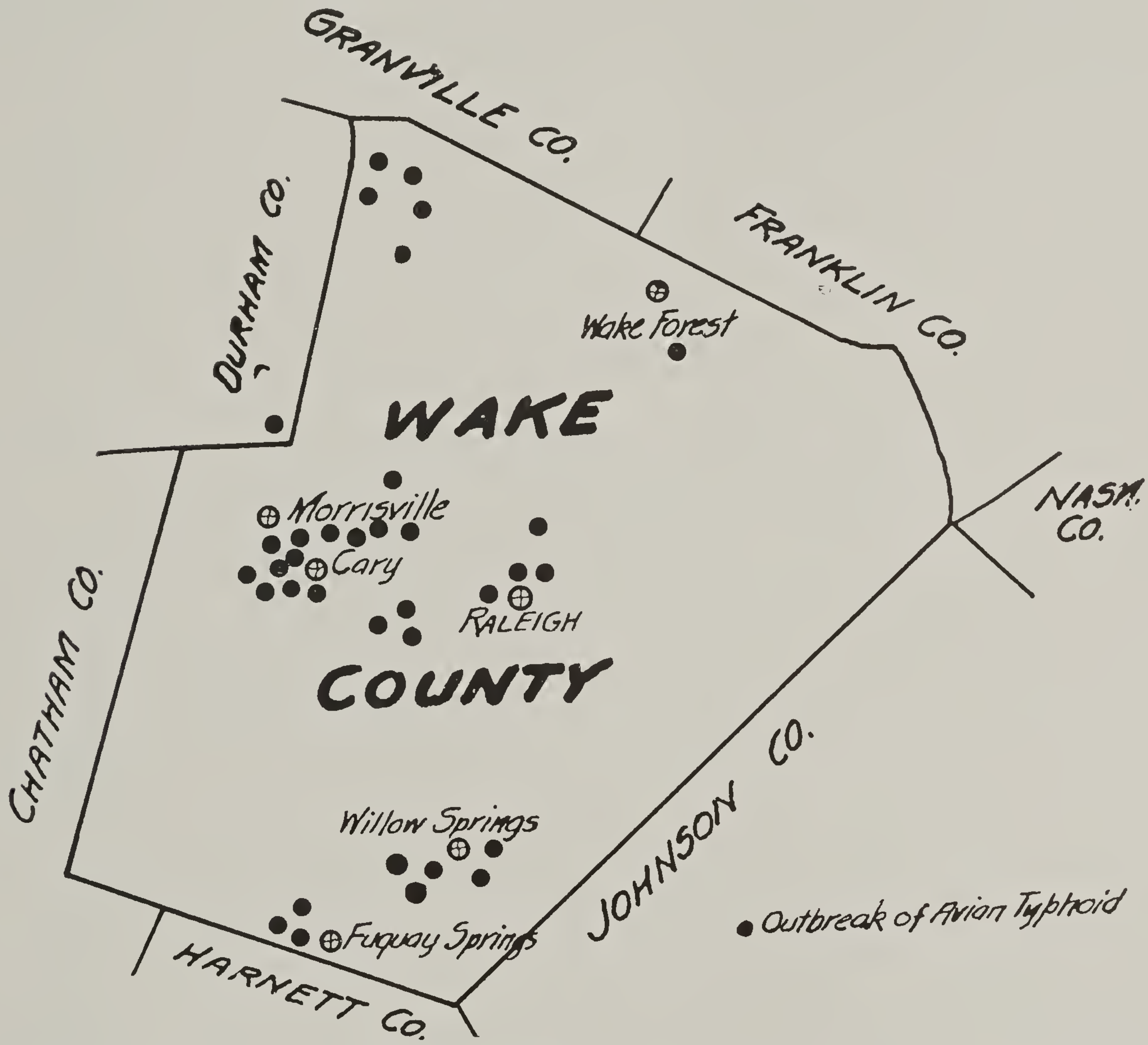

FIG. 2.—PREVALENCE OF FOWL TYPHOID IN WAKE COUNTY, N. C. EACH DOT REPRESENTS AN OUTBREAK

In North Carolina, since the inception of these studies, 55 outbreaks have been encountered in which the causative organism has been recovered, or in which reliable data could be secured. The distribution of the disease in the state is shown in Figure I, demonstrating that avian typhoid is prevalent from the mountains to the coast.

In Wake County, in which the State College is located, and where it was possible to make intensive investigations, 35 outbreaks were studied, involving 2,536 birds, and incurring the death of 545 members of the affected flocks. The monetary value of birds dying in these outbreaks would approximate $700 and does not include the possible mortality had prophylactic vaccination not been resorted to. The distribution of the disease in the county in shown in Figure 2.

The investigators have reason to believe that the incidence of avian typhoid in Wake County is typical of that throughout the state, and probably represents that of the section.

HISTORICAL RESUME*

Baroneo, a celebrated Lombardy (Italy) physician, appears to have been among the first to seriously consider the nature and cause of serious typhoid-like epidemics which had been noted by poultry raisers even before his time. Together with Miocchi and Brugnatelli, he examined with considerable care the extensive epidemics which occurred among poultry in Lombardy betweeen the years of 1789 and 1798, and which were commonly referred to by the poultrymen of the Turin district under the name "calcinaccio," and by the Piemontese as "Causinera." Somewhat later, similar epidemics in Piemonte were studied by Brugnone and Toggia. In view of the lack of bacteriological knowledge and technique in these early years, it is, of course, clear that there are few data, save the description of the clinical features of the malady, to serve as a basis of connection with cholera-like diseases of poultry as we know them at the present day; and it is therefore impossible definitely to say whether they represented true fowl cholera or some other disease. But in view of the circumstances that when advances in bacteriological knowledge and technique finally did permit a certain degree of differentiation, the non-cholera type of infection appeared to be the prevailing malady of the Italian provinces, and it can perhaps be justifiably assumed that, even in earlier years, it was a well established poultry scourge in these sections of southern Europe, whence much of our own poultry has been derived.

Lemaistre was undoubtedly the first to refer to one of the epidemic diseases of poultry as typhus or typhoid ("Epizootie (typhus) des Gallinaces"). He observed extensive epidemics in the Haute-Vienne in 1832, 1849, and again in 1864. He gives an excellent description of the symptoms and pathological manifestations of the malady, and speculates regarding the probable cause. He considers in turn the feeding, the housing, the range conditions, and the possibility of climatic alterations, but sees in them no causal agency. As might have been expected in those early days, he finally arrives at the agency of miasms. He reports that he, himself, felt unwell after the autopsy of several sick birds in a small close room, and believed it might have been due to the absorption of miasms. He considered the possibility of danger to man from eating the flesh of diseased fowls, but states that the peasants, when observing the birds to become sick, commonly kill and eat them; and he concludes no illness has probably resulted, although he recommends otherwise. It was several years after Lemaistre's observations that the actual causative agents of the disease was recognized.

In 1877, Joannes again described the so-called "typhus" of birds. He differentiated between the "local enzootic typhus (simple septicaemia)," in which the malady did not appear to extend itself, and the epizootic typhus which became easily disseminated "through villages, departments, and even provinces." The bacteriology of these disease types does not receive ample consideration.

Megnin, in 1877, studied certain epidemics in poultry which he undertook to separate from cholera, and which he called "Septicaemia." This view of a disease distinct from cholera is in harmony with the view of most of the Italian School at that time, though opposed by Perroncito. Lucet believed the epidemic of Megnin corresponded in every respect with those of Lemaistre and Benion.

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* Unless otherwise indicated this Historical Resume is taken from the comprehensive review presented by Hadley in Bulletin 174 of the Rhode Island State College, May, 1918.

In the year 1878 bacteriological technique had been somewhat advanced, and in this year, Perroncito presents under the term "epizootic typhoid" (following the terminology of Lemaistre) results of the first scientific study of the disease which we at present must consider as typhoid of fowls, although Perroncito later came to the conclusion that the disease was fowl cholera.

The decade following the work of Perroncito was a period devoted particularly to the study of fowl cholera, and interest in other cholera-like epidemics correspondingly waned, although it can scarcely be doubted that even at this time much confusion existed as to the real nature of the diseases that were studied. This was the period of the first definite knowledge of the organisms later to be recognized as the hemorrhagic septicaemia group, and it was marked by the brilliant researches of Toussaint and Pasteur.

Eberth, in 1880, described under the heading of "affection diarrheique des perroquets" a malady characterized by great weakness and convulsions. In the blood was found a "special type of round organism." The author believed the disease corresponded with the dysentery of parrots previously described by Percheron, and by Benion and Megnin.

In 1884 Petri recorded an epidemic which attacked geese, ducks, and hens. The disease took the form of a septicaemia, the blood revealing great numbers of small bipolar organisms. Inoculations showed the culture to be pathogenic for mice, rabbits, and pigeons. Guinea pigs were refractory. The author believes the disease is similar to that previously studied by Gaffky, and was due to the "bacillus de la septicemie experimentale du lapin."

In 1888 Cornil and Toupet studied an epidemic among geese, spoken of as "cholera des canards." This malady was caused by an organism simulating that of fowl cholera. The authors were able to show, however, that while the causative agent of fowl cholera was pathogenic for geese, rabbits, fowls, and pigeons, the causative agent of goose cholera was pathogenic in average doses, for geese only, and for rabbits in large doses only. They thus concluded that the organisms were different, though related, and that the virus of goose cholera had become fixed at a degree less virulent than that of fowls. Lignieres (1900), however, believed that the organism of these authors could not be placed in the Pasteurella group.

The first reference to the typhoid-like diseases of birds in which the organism is given a definite name is that of E. Klein, in 1889, who presents the details of an extensive epidemic among fowls in Kent, England. The mortality which developed in March, 1888, eventually reached over 400. With the exception of the leukemic condition, the clinical symptoms and the post mortem findings agree well with those presented by Moore (1895) for his "infectious leukemia" of fowls. Klein's description of the causative organism (Bacillus gallinarum), its occurrence in the blood, morphology, staining characteristics, inoculation experiments and cultural features in the few medias available for bacteriological use at that time, make it appear highly probable that he worked with the same bacterium which Moore described several years later under the head of Bacterium sanguinarium, the causative agent of infectious leukemia in the United States.

Klein also studied in 1889, an epidemic among Scottish grouse. From these cases he obtained a small motile rod measuring 0.6 to 1.8 microns, which he termed B. scoticus.

In 1889 Reick reported an epidemic among canaries in Dresden. All cases showed intestinal catarrh and liver changes. A polar staining organism was isolated from the organs. The number of bacteria in the blood was small. The organisms were mostly in pairs and showed marked motility. The author points to a close resemblance between this disease and fowl typhoid, and refers especially to the sulphur yellow necrosis at the site of inoculation.

Typhoid in canaries had not, however, previously been reported. He finally concludes that his organism is a type sui generis, morphologically like fowl typhoid but biologically different.

Lucet, in 1891, described under the title of "epizootic dysentery of fowls," a malady which seems to differ in no essential manner from the so-called fowl typhoid, and from the disease reported by Klein and earlier writers. The disease was reported to attack birds of all ages, but chiefly fowls one year old. Among the prominent symptoms, Lucet reported pale comb, yellowish or reddish diarrheal droppings, exudation from mouth, and a rise in temperature of one to two degrees. Death usually came nine to thirteen hours after the appearance of the symptoms, though some cases endured for twenty days and some birds recovered—then manifesting a temperature two to three degrees subnormal. The pathological anatomy was characterized by fatty drops in the blood, lungs hyperemic, serous pericardial exudate, liver enlarged and friable with hemorrhagic extravasations, gall bladder distended, and kidneys congested. The organism isolated quickly lost its virulence, although it increased by passages through hens. Rabbits were susceptible only through intravenous injection; pigeons could be infected, at least by a subcutaneous inoculation, but guinea pigs were refractory. The organisms were present in the blood only in small numbers.

Lucet makes a comparison between his bacterium and the B. gallinarum of Klein, and concludes that they are different species.

Foth, in 1892, reported an epidemic among fowls from which he isolated a bacillus which in its motility, staining and cultural features resembled **B. typhosus.** It differed in this respect that it formed gas. The blood and organ tissues swarmed with the organisms. Inoculation into rabbits subcutaneously resulted in local reaction only. Young rabbits, however, were killed by intravenous inoculation. Pigeons and hens inoculated subcutaneously were refractory, but gave intense local reaction. No other details of the biochemical features of the culture are presented.

In 1894 Perroncito studied an infection in Piemonte from which he isolated an organism about twice the size of the fowl cholera bacterium. Aside from the fact that it did not liquefy gelatin and produced only a tumefaction at the site of the injection in fowls, no other data was presented. Perroncito believed that this disease, as well as the epidemic which he reported in 1878, was distinguishable from the true fowl cholera. It is important to observe, however, that by the year 1899, Perroncito's view of this matter was changed. In sittings of the Academy of Medicine of Turin, held on May 19 and 26, 1899, he opposes the views of Mazza, Abba, and Foa, that the then present epidemics among fowls in northern Italy were not fowl cholera. At this time he states his belief that the epidemics previously studied by him in 1878 and 1894 were really examples of fowl cholera, although he did not previously accept this view. This conclusion is opposed by Mazza, Abba and Foa, who adhere to the view that the then prevailing epizootic, as well as maladies studied earlier, differ from the actual fowl cholera in many respects, relating to the causative agent, and the pathological conditions revealed at autopsy, as well as in the pathogenicity for hens, pigeons, and laboratory animals.

In 1894 Loir Duclaux studied in Tunis an epidemic among fowls from which they isolated a bacillus which they termed B. avium. The organism was a short gram-negative bacillus with rounded ends, which grew well on ordinary culture media. Only a meagre presentation of the cultural features occurs.

In 1894 Lignieres examined a number of hens which died showing diarrhea but no other symptoms. At autopsy he observed generalized inflammation of the intestinal tract, and a quantity of serous fluid in the body cavity, together with enlargement of the spleen and liver. From the spleen he isolated a culture which appeared to be of the B. coli type. Hens resisted intravenous, subcutaneous and intramuscular injection of the culture. Pigeons succumbed to infection by all of these methods. Subcutaneous inoculation of rabbits resulted in abscess formation only, but intrapleural and intraperitoneal inoculations were fatal in 24 hours. The same was true for guinea pigs.

Leclainche, in 1894, reported on a disease of ring doves observed in the autumn of 1893. The clinical symptoms involved somnolence, weakness, diarrhea, and the turning of the head and neck to one side. The clinical picture showed a congested intestinal mucosa with injection of the intestinal vessels. There were also signs of general septicaemia. The liver was usually greatly enlarged, the spleen friable, but the lungs normal. From the tissues of the birds dying of the disease the author was able to isolate small ovoid, non-motile, non-liquefying, gram-negative rods, showing upon staining a clear space in the center. In the blood the rods appeared much shorter, and in cultures they frequently had the aspect of diplococci.

Moore (23), in 1895, in a comprehensive study of "infectious leukemia" of fowls, brought out the fact that avian typhoid was present in the domesticated birds of America. These studies included the morbid anatomy, clinical studies of the disease, and especially emphasized the changes in composition of the blood. Moore isolated the Bacterium sanguinarium in outbreaks investigated, and designated it as the causative agent in fowl typhoid.

In 1895 Kern studied what he regarded as a new infectious malady of canaries in Budapest. The epidemic had its inception in the introduction of two new male birds. During the outbreak, 25 to 28 females died, followed by the two males. Kern does not describe the organism isolated by him with sufficient detail to make its nature clear. He regarded it as the agent of hemorrhagic septicaemia, but his photographs of the bacillus suggest B. gallinarum, the causative agent of fowl typhoid. The bacillus was not pathogenic for fowls, and few were found in the blood, but many in the tissue cells.

Dawson, in 1898, reported an epidemic which caused serious losses among poultry in Maryland, and which was assumed to be fowl cholera. His descriptions of the disease agrees in essential features with those given by Moore for infectious leukemia. The organism which he described is similar to the bacillus of Klein and the bacillus of Moore.

In 1900 Pagliani, in the name of Dr. Mazza, reported in the Academy of Medicine at Turin the results of a study of twenty-seven cases of fowl typhoid-like diseases in poultry. Among these he reported the isolation of the "typhoid bacillus" twenty-two times, and the bacillus of fowl cholera five times. This appeared to be the ratio of these disease types in the Italian provinces at that time. It now seems clear, however, that more than one bacterial form may have been included in Pagliani's twenty-two fowl typhoid cases.

During the years 1900 and 1901 Centani and Savonizzi, Lode and Gruber and Krauss, did considerable work of the disease designated as "exudative typhus," probably due to a filterable virus. This was followed by the illuminating studies by Maggiora and Valenti on the same subject.

Curtis (3), in 1901, reported an extensive outbreak of contagious disease in poultry, from which Smith isolated the Bacterium sanguinarium.

Riemer, in 1904, reported on an epidemic among geese in the vicinity of Mecklenburg, from which he isolated a non-motile, gramnegative rod occurring predominately in pairs, but sometimes in filaments. Riemer's description of the pathological changes, as well as the causative agent, leaves little doubt that his case involved an infection with an organism of the fowl typhoid type.

Lignieres and Zabula in 1905 described epidemics among poultry in Buenos Ayres, which they referred to as "Salmonellosis." Guinea hens, ducks, and pigeons were not affected. The usual fowl typhoid symptoms appeared, accompanied by a temperature of 42 to 43 degrees C.

Smith and Ten Broeck (27), in 1915, studied the agglutinative affinities of Bacterium sanguinarium with B. typhosus, together with its toxin producing properties, and its relation to B. pullorum. These investigators prefer to regard Pullorum and Sanguinarium as separate types.

Rettger and Koser (26), in 1917, made a comprehensive comparative study of B. pullorum and B. sanguinarium, bringing out the following facts: (1) Bacterium pullorum and Bacterium sanguinarium constitute two separate types bearing a specific relationship to the disease which it had been associated with in the past; (2) There is a differentiation in carbohydrate reaction; (3) Differentiation is possible by the methyl red test, sanguinarium being methyl red positive in 1 per cent maltose bouillon, while pullorum is negative; (4) The disease attacks fowls of all ages in experimental inoculation, and while pullorum infection is natural with chicks in the field, sanguinarium is of little importance as a cause of chick loss under natural conditions.

Taylor (30), in 1916, reports an outbreak of avian typhoid in California, and makes a study on morbid anatomy, blood changes, susceptibility, and modes of infection. In accordance with Rettger and Koser, Taylor concludes that fowl typhoid is a specific disease caused by the Bacterium sanguinarium, and that though resembling Bacterium pullorum biologically, they are distinct diseases.

In 1917 Goldberg (8) made a detailed study of the fermenting properties of pullorum and sanguinarium, and arrived at a means of differentiation through gas production and other biological activities.

Hadley (10), in 1919, presented an article on the differentiation between fowl typhoid and fowl cholera, in which he brought out differences from the following standpoints: (1) Nature of the disease and pathological alterations in the organs and tissues; (2) The bio-chemical features of the causative agents; (3) Their toxicity or virulence; (4) Their sereological reactions; (5) Their immunological reactions.

Hadley, in 1919, goes into the colon typhoid intermediates as the causes of diseases in birds, assigning the causative organism of avian typhoid its place in this group of organisms, and entering into a detail description of the methods of differentiating members of the group.

In 1919 Muslow (24) presented a very complete study on avian paratyphoid bacilli, going into the biological activities of B. avisepticus, B. sanguinarium, and B. pullorum. Muslow also presents agglutination studies and experimental inoculation data, together with differentiation information between B. typhosus and the three organisms mentioned above.

teHennepe and Van Straaten (33) at the World's Poultry Congress at The Hague in 1921 presented a paper on Fowl Septicaemia, in which Klein's disease (avian typhoid) occupied a prominent part. These investigators dwelled on the prevalence of the disease in The Netherlands, the serology and morbid anatomy, and maintain that the disease can be combatted successfully by means of "hygenic measures, vaccination, and serum therapy."

d'Herelle (4), referring in The Bacteriophage on the relation of this phenomena to avian typhoid, goes into this disease as to its prevalence in France; its close relationship to other organisms of the colon-typhoid group, and dwells at considerable length on the role of the bacteriophage in the natural immunity shown by some birds in outbreaks of this disease.

Truche (31) in 1923 studied avian typhoid in France, stressing the causative oraganism, morbid anatomy, susceptibility of laboratory animals, and the differential diagnosis between avian typhoid and fowl cholera. Truche also touches somewhat on the differentiation between pullorum and sanguinarium.

Truche and Staub (32) in a report at the World's Poultry Congress at Barcelona, Spain, in 1924, reports that the researches of the Pasteur Institute in relation to poultry diseases is pointing toward the development of polyvalent vaccines against avian typhoid.

In 1923 Kaupp and Dearstyne (15) reported the detail study of a hen which, under field conditions might have been a carrier of avian typhoid, and stress the fact that such birds are a possibility in the recurrence of the disease in an infected flock. This study was followed by a detailed study of morphological and physiological characteristics of E. sanguinaria, with graphic presentations of the spread of the disease in the field (16). In February, 1924, a case was reported in which a bird was artificially infected with avian typhoid, the organism being recovered from the blood current of the bird and used to infect a second bird. The first bird recovered and the second succumbed. This study brought out the fact that one bird had sufficient natural immunity to throw off the disease, while the second did not. In 1924 the North Carolina station reported a comparative study of the European strains of avian typhoid with those of North America, no outstanding differences being found in the biological activities, or in the general character of the disease produced by these various strains. A final article on the differentiation between avian typhoid and avian cholera (18) was published showing morphological and physiological differences; in the causative organisms, differences in duration of the diseases and in clinical symptoms. Hemotological studies were also included.

In 1924 Bushnell and Patton (2) presented the various phases of the use of vaccines in poultry husbandry, bringing out the practicability of their use in controlling avian typhoid and other avian diseases.

THE CAUSATIVE ORGANISM

The causative organism of avian typhoid received its first designation from Klein in 1889, the organism being known as Bacillus gallinarum, or Klein's bacillus. Moore, in 1895, isolated an organism from an outbreak which he called "Infectious Leukemia," and designated the causative organism Bacterium sanguinarium. This designation was adopted by Moore with reference to Migulas classification of bacteria, based on the absence of motility, the specific name sanguinarium being suggested because the most conspicuous lesion in fowls affected with the disease was in the blood. Moore's organism and that of Klein later proved to be the same. Pfeiler and Rehse, in 1913, designated what probably was the avian typhoid organism as Bacterium typhi gallinarum alcalifacians.

In 1917-20 the Society of American Bacteriologists adopted the classification of Bergey and his collaborators, and designated the causative organism of avian typhoid as Eberthella sanguinaria (Moore), by which name it is now recognized by the American School.

MORPHOLOGICAL STUDIES

Eberthella sanguinaria (Moore) is a non-motile, gramnegative organism, showing no capsule, endo spores, or irregular forms. The size of the organism varies, the usual dimensions ranging from 1 to 2 microns in length, and from 0.5 to 0.8 microns in width. The Brownian movement is well marked. The ends are rounded; the organism stains readily with most analine stains, the peripheral structure taking the strain most heavily. Though the organism is gram negative, this stain is not readily taken. Cases have been noted when staining directly from the blood that the organism had a tendency toward bipolar staining, thereby rendering the possibility of confusion with members of the pasturella group. In direct isolations there is a tendency toward coccoid shape in formation. Continuous cultivation on artificial medium causes the organism to loose the property of peripheral staining.

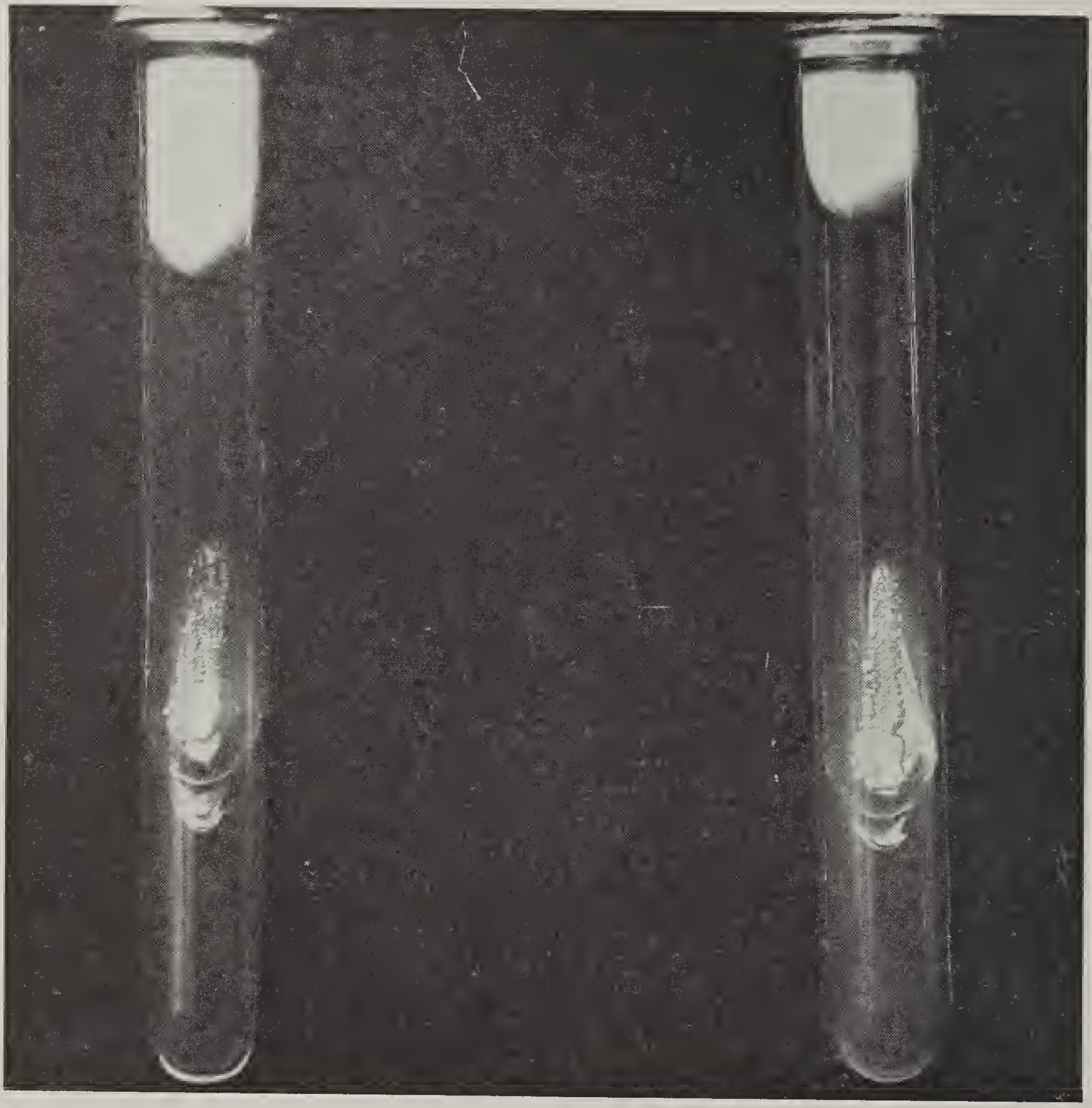

FIG. 3.—EBERTHELLA SANGUINARIA (MOORE) ON AGAR SLANT

NOTATION.—Studies on the causative organism, except where otherwise indicated, are the observations of this laboratory. The technique is that of Pure Culture Study of Bacteria, as adopted by the Society of American Bacteriologists.

CULTURAL CHARACTERISTICS

Agar Slant.—The growth on agar slant is abundant, beaded, raised, dull, smooth, opaque, non-chromogenic, and gives off a decided pungent odor after 24 hours incubation. The growth is semi-viscid and the medium remains unchanged. Sufficient growth for observation is usually obtained after 16 to 18 hours.

Agar Colonies.—Colonies on agar plates make rapid growth, are punctiform in form, smooth, raised, entire and finely granular in consistency.

Gelatin.—In gelatin stabs the growth is uniform along the site of stab, beaded, non-liquifying, with the medium remaining unchanged.

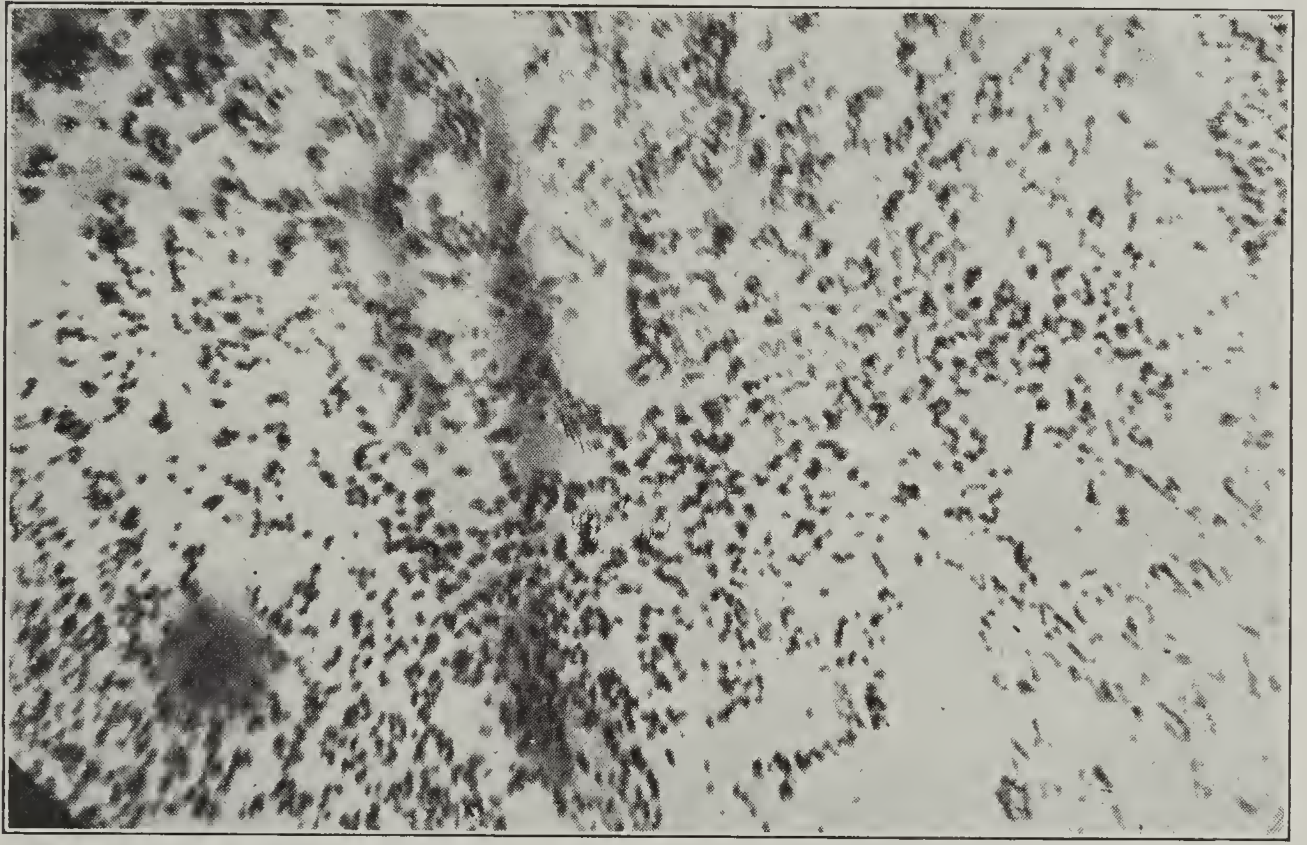

FIG. 4.—PHOTOMICROGRAPH OF EBERTHELLA SANGUINARIA

Potato.—On potato there is usually a moderate growth, yellowish-brown is color in 24 hours.

Nutrient Broth.—There is no surface growth; moderate clouding in 24 hours emitting a distinct penetrating odor, and producing a scanty flaky sediment.

Temperature Relations.—The optimum temperature for growth of E. sanguinaria is 37½ degrees C. The maximum temperature at which suitable growth is secured is 44 degrees C., while a fairly good growth is produced at 18 degrees C. in 72 hours. The thermal death point of the organism is 62½ degrees C. for ten minutes, and it resists dry heat on glass rods for 5 minutes at 75 degrees C., but is killed at the same temperature when heated for 10 minutes.

Relation to Reaction of the Medium.—On agar, the optimum hydrogen ion concentration is about pH 6.4. The organism has a high tolerance to organic acids, suitable growth being obtained on agar acidified with malic acid, with oxalic acid, and with formic acid between the ranges of pH 4.8 and 7.0.

Production of Indole.—There is a slight production of indole in some instances with the Salkowski test, but no reaction with the vanillin test, nor with the Ehrlich method. The Salkowski reaction, however, is not consistent. Moore (23) obtained a distinct reaction with the Kitasato method, but Smith and Ten Broeck, Muslow, and Hadley failed to obtain positive tests for indole.

Chromogenisis.—The organism is non-chromogenic on agar and gelatin. There is usually a yellowish-brown coloration of the growth on potato.

Relation to Oxygen.—On glucose agar shakes, there is a fairly heavy surface growth present in 24 hours.

Milk.—Milk is rendered acid during the first few days, after which alkali formation is noted. The reaction is variable, especially in the time needed to form alkali. The tests were conducted with 1.0 per cent of a 0.5 per cent solution of brom cresol purple, and with litmus solution.

Nitrate Reduction.—Heavy reduction of nitrates was noted on nitrate agar and in nitrate broth after 48 hours of incubation. There was no gas formation. This finding is opposed to that of Hadley (10), but in accordance with that of Rettger and Koser (26).

Hydrolysis of Starch.—There is a slight diastatic reaction on starch agar plates in 48 hours, the zone of reduction varying in size, but being small on the average.

Effects of Disinfectants.—E. sanguinaria is killed in dilutions of 1:1000 phenol, and in 1:20,000 $HgCl_2$.

Effects of Dessication.—The organism on glass rods immersed in bouillon cultures retains its vitality in the dark up to 89 hours, but loses it in the light in less than 30 hours.

Longevity.—E. sanguinaria will live in distilled water and in tap water in the dark for over 20 days, but is killed in the light in the same mediums in less than 24 hours.

Fermentation Studies.—Fermentation studies were carried out with 1% of the carbohydrate or higher alcohol added to beef extract broth according to Methods of Pure Culture Study. In Table I the number of strains is indicated. These strains included those from various sections of the United States, from England, France, Holland and Belgium, and should be representative of the specie. The inoculations were incubated for 5 days at 37½ degrees C.

Table I

EFFECTS OF E. SANGUINARIA ON THE VARIOUS CARBOHYDRATES

Carbohydrate or Higher Alcohol	*Number of Strains*	*Gas Prod.*	*Orig. pH*	*pH After 5 Days (Average)*	*Reaction*
Arabinose	55	0	7.0	6.4	Acid
Dextrin	53	0	7.0	6.4	Acid
Dextrose	23	0	7.0	6.6	Acid
Dulcite	54	0	7.0	6.6	Acid
Galactose	48	0	7.0	6.4	Acid
Inulin	52	0	7.0	6.4	Acid
Lactose	23	0	7.0	7.2	Alkaline
Levulose	54	0	7.0	6.4	Acid
Mannose	21	0	7.0	6.2	Acid
Maltose	25	0	7.0	5.8	Acid
Saccharose	54	0	7.0	6.4	Acid
Xylose	55	0	7.0	6.2	Acid

As indicated, no gas was formed in any of these fermentation tests. This result agrees with all available scientific literature on this subject as to the lack of ability of E. sanguinaria to form gas in any known carbohydrate or higher alcohol. Rettger and Koser (26) tried experimentally to bring about gas production with sanguinaria by altering the compositions of medias, but were unsuccessful.

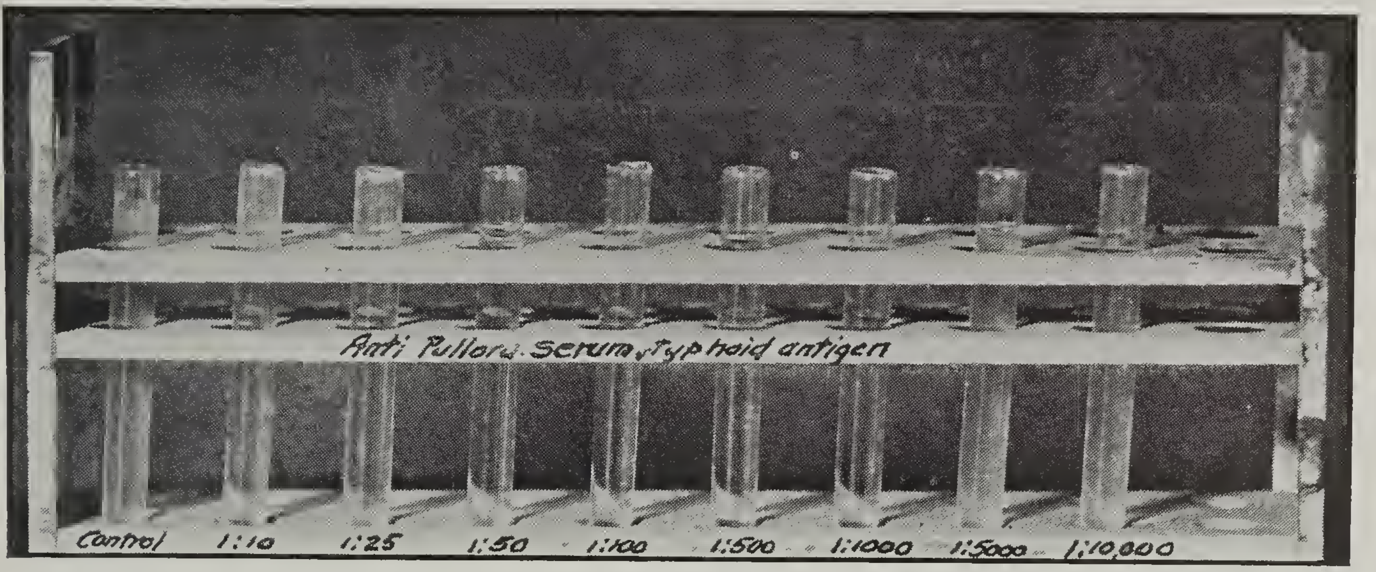
Anti Pullora Serum, typhoid antigen
Control
1:10
1:25
1:50
1:100
1:500
1:1000
1:5000
1:10,000

FIG. 5.—CROSS-AGGLUTINATION. ANTI-PULLORA SERUM V. TYPHOID ANTIGEN

The tendency of sanguinaria to produce a slight alkalinity in Lactose is in agreement with the work of Taylor, Muslow, Goldberg (8) and Rettger and Koser.

With the exception of the reaction in saccharose the results obtained in the carbohydrate reactions agreed with those of Rettger and Koser, Hadley, Muslow, Moore, and Goldberg. After 5 days of incubation we were unable to demonstrate neutrality in saccharose with tests on 54 strains. In some instances, however, the reaction produced was nearer neutrality than the average would indicate.

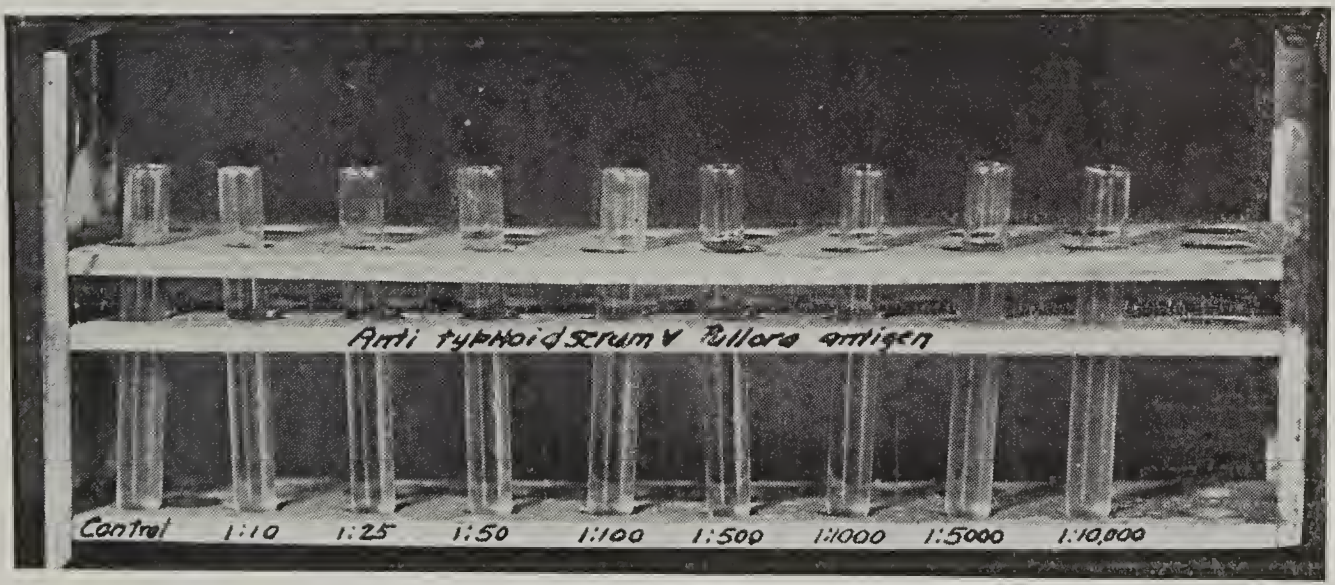
Anti typhoid Serum V Pullora antigen
Control
1:10
1:25
1:50
1:100
1:500
1:1000
1:5000
1:10,000

FIG. 6.—CROSS-AGGLUTINATION. ANTI-AVIAN TYPHOID SERUM V. PULLORA ANTIGEN

Methyl Red Test.—E sanguinaria is methyl red positive in Maltose broth after 48 hours incubation.

Pathogenicity.—The domestic fowl, turkey, guinea, pigeon, duck, and geese are susceptible to E. sanguinaria, these birds being susceptible to laboratory infection. Sparrows are susceptible to subcutaneous inoculation. White rats and mice are mildly susceptible to subcutaneous inoculation, but succumb to intraperitoneal injection within 5 days. Guinea pigs succumb to intraperitoneal inoculation, but resist subcutaneous injection. Rabbits succumb to small doses of the organism.

Muslow reports rabbits, guinea pigs, cats, rats, mice, sparrows, pigeons and chickens non-susceptible to laboratory cultures of B. pullorum and B. sanguinarium when administered by the mouth. This does not agree with the findings of this laboratory in regard to experimental infection of chickens and pigeons.

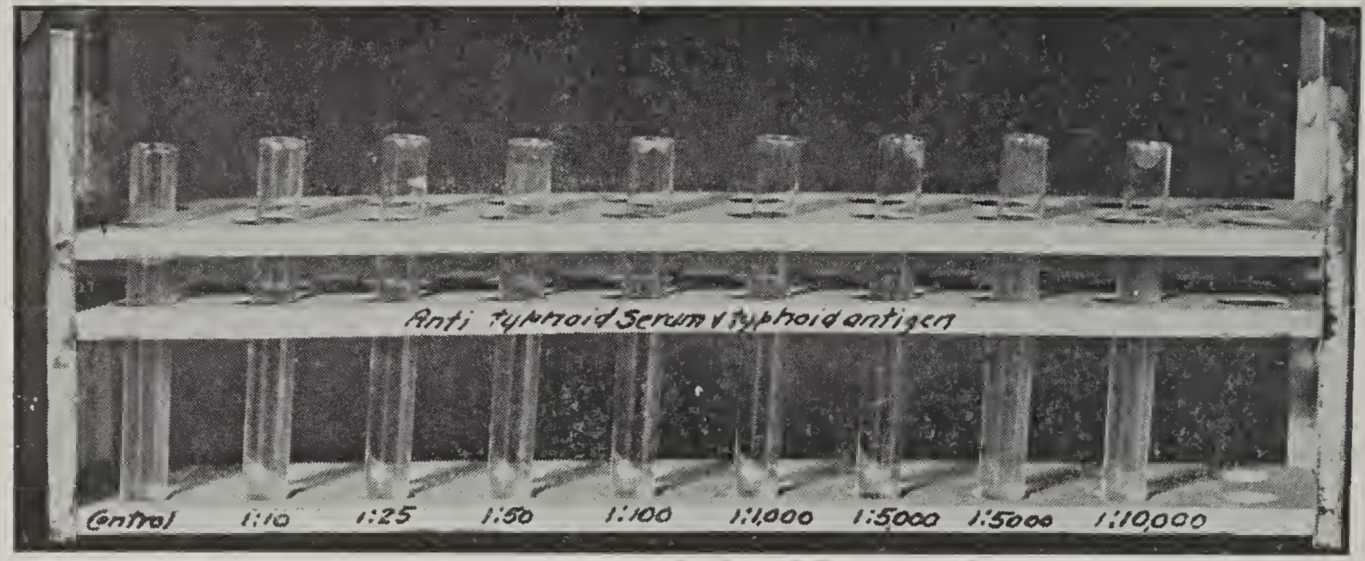

FIG. 7.—CROSS-AGGLUTINATION. ANTI-AVIAN TYPHOID SERUM V. AVIAN TYPHOID ANTIGEN

Agglutination.—Agglutination tests with B. typhosus, Paratyphoid A, Paratyphoid B, S. pullora and E. sanguinaria show an antigenic relationship between these organisms. This phenomena was first observed by Smith and Ten Broeck. The results of these agglutination tests are shown in Table II, and are graphically presented in Figures 5, 6, 7. The anti-pullorum serum used in these tests was secured through the courtesy of Dr. Rettger, and the anti-avian typhoid serum was developed in this laboratory. The results obtained in these tests approximate the findings of Smith and Ten Broeck, Rettger and Koser, Hadley, Muslow, and Krumweide and Kohn.

Table II

Agglutination With Immune Serum to E. Sanguinaria Titer 1:5000

	Control	1:10	1:25	1:50	1:100	1:500	1:1000	1:5000	1:10000
B. typhosus	—	++++	++++	++++	++++	++++	++++	—	—
B. paratyphosus A	—	++++	++++	++++	++++	++++	++	—	—
B. paratyphosus B	—	++++	++++	++++	++++	+++	+	—	—
S. pullora	—	++++	++++	++++	++++	++++	++++	—	—

Agglutination With Immune Serum to S. Pullora Titer 1:1000

B. typhosus	—	++++	++++	++++	++++	++++	++++	—	—
B. paratyphosus A	—	++++	++++	++++	++++	++++	++	—	—
B. paratyphosus B	—	++++	++++	++++	++++	++	—	—	—
E. sanguinaria	—	++++	++++	++++	++++	++++	++++	—	—

FIELD STUDIES

The studies of the disease in the field presents striking opportunities for observation of this contagium under natural conditions. Here enter nature's factors such as sunshine, the air, rain, snow, and changeable conditions of temperature with a possible range of 100 in midsummer to near or below zero in winter. There also exists in the field such conditions of contact as are not present in the studies made under artificial infection conditions.

The influence of nature's forces of spreading or retarding the spread of this disease in the field is problematical. The germicidal effects of the direct rays of the sun are undisputed. Many pathogenic organisms are resistant to dessication, others have not these powers of resistance. This factor involves the phenomena of sporation which is not common to the group of organisms pathogenic to the domestic fowl. The chances of the spread of avian typhoid through the air seems remote. Salmon (28) in his original studies on fowl cholera in 1880 arrived at the conclusion, after a series of experiments, that the chances of the dissemination of this disease through the air was negligible. During the artificial infection work at this station, birds were left in confinement in the same room in adjacent coops, though not in actual contact, with other birds in all stages of avian typhoid. Such well birds failed to show any symptoms of the disease. If the air was a factor of transmission, it is highly probable that some takes would have occurred, the likelihood of infection being enhanced because under conditions of confinement the natural resistance of birds is at a low ebb.

It is natural to assume that avian typhoid might be transmitted through surface washing. Heavy rains will drain infected soil, and with it many virulent organisms incorporated with small particles of debris. If the topography of the land is favorable, heavy rains are probable factors of disseminating this and other avian diseases. The ability of E. sanguinaria to live and retain its virulence in water in the dark for 20 days, and in the same medium in the sunlight for less than 24 hours (16) admits of the possibility of the organism being transmitted from farm to farm through the medium of small brooks, and the overflow of the same in rainy season.

Gwatkin (9) dwells on the spread of avian typhoid in the following:

"Infection is spread by sick or recently recovered birds introduced into the flock. It may also be introduced by the boots, clothing, utensils or on animals that have been on infected premises. The droppings of affected birds contain enormous numbers of the germs which are picked up by the others while eating and drinking. In this way the infection spreads very rapidly."

Pfeiler and Standfuss (25) maintain that the disease has an acute and a subacute stage, and that it is transmitted from bird to bird by cohabitation.

Curtis (3) stressed the introduction of the disease into the flock through the medium of new birds purchased, and advocated the isolation of all new birds until the status of their health could be definitely determined.

d'Herelle (4) in commenting on the spread of avian typhoid says:

"The establishment of a new focus begins by the importation of the organism from an infected region either through the agency of a flock of sheep, herd of cattle, or by horsemen. The disease rages for a few days on a farm, passes to a neighboring farm, and then extends rapidly into the surrounding villages."

The field observations of this department pointed toward the fact that the mechanical transportation of the disease from flock to flock was of paramount importance. It is probable that the English sparrow and other wild birds are potential conveyors of this contagium. Positive data on the sparrow as a mechanical means of conveyance of avian typhoid is difficult to obtain. It is a matter of common occurrence for sparrows to utilize the feed troughs and drinking containers in poultry yards occupied by flocks in all

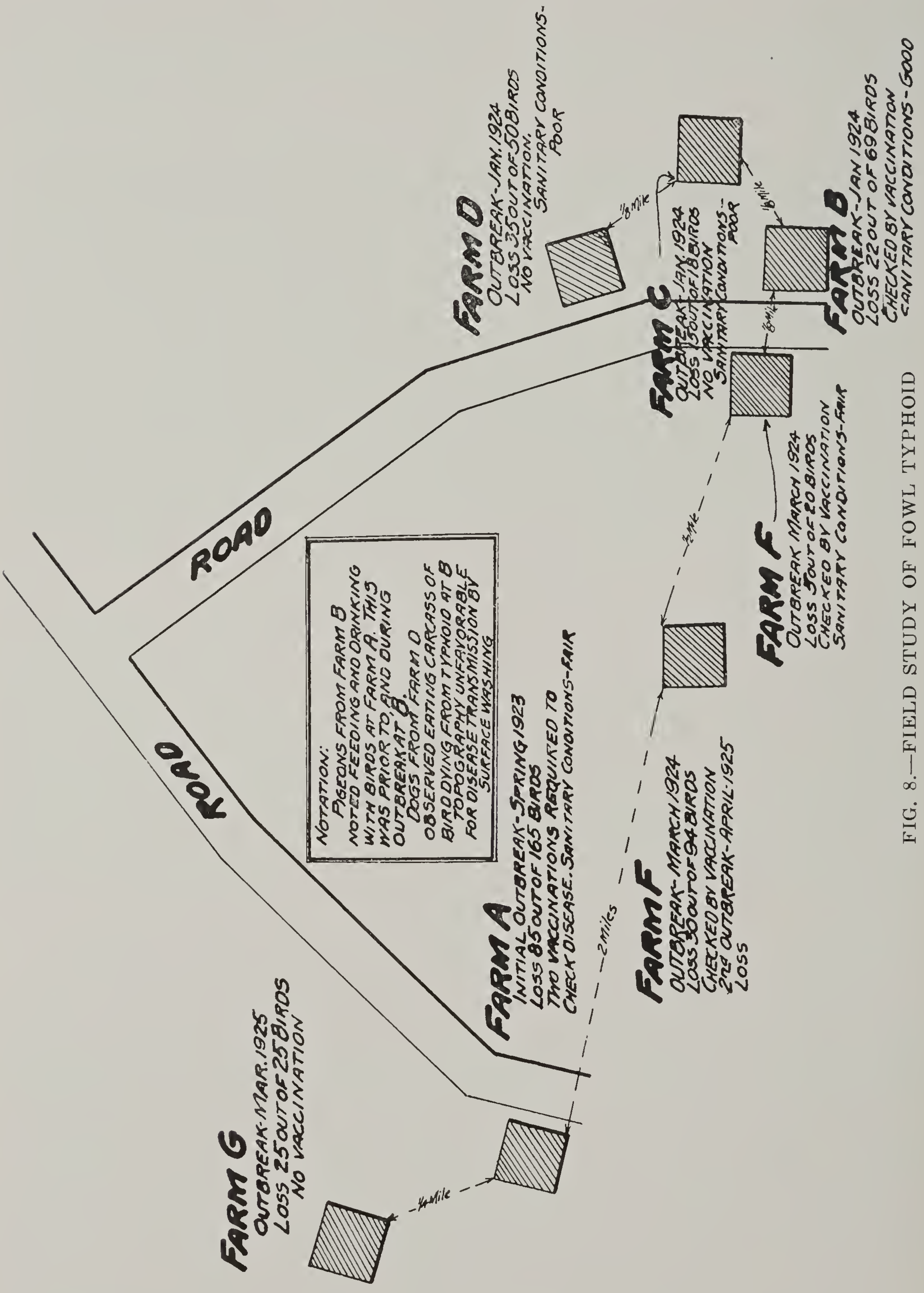
FARM D
OUTBREAK-JAN.1924
LOSS 35 OUT OF 50 BIRDS
NO VACCINATION.
SANITARY CONDITIONS-POOR
1/8 Mile
FARM C
OUTBREAK-JAN.1924
LOSS 15 OUT OF 18 BIRDS
NO VACCINATION
SANITARY CONDITIONS-POOR
1/8 Mile
FARM B
OUTBREAK-JAN 1924
LOSS 22 OUT OF 69 BIRDS
CHECKED BY VACCINATION
SANITARY CONDITIONS-GOOD
1/8 Mile
FARM F
OUTBREAK MARCH 1924
LOSS 5 OUT OF 20 BIRDS
CHECKED BY VACCINATION
SANITARY CONDITIONS-FAIR
3 Mile
ROAD
ROAD
NOTATION:
PIGEONS FROM FARM B
NOTED FEEDING AND DRINKING
WITH BIRDS AT FARM A. THIS
WAS PRIOR TO AND DURING
OUTBREAK AT B.
DOGS FROM FARM D
OBSERVED EATING CARCASS OF
BIRD DYING FROM TYPHOID AT B
TOPOGRAPHY UNFAVORABLE
FOR DISEASE TRANSMISSION BY
SURFACE WASHING
FARM A
INITIAL OUTBREAK-SPRING 1923
LOSS 85 OUT OF 165 BIRDS
TWO VACCINATIONS REQUIRED TO
CHECK DISEASE. SANITARY CONDITIONS-FAIR
2 Miles
FARM F
OUTBREAK-MARCH 1924
LOSS 30 OUT OF 94 BIRDS
CHECKED BY VACCINATION
2nd OUTBREAK-APRIL-1925
LOSS
FARM G
OUTBREAK-MAR.1925
LOSS 25 OUT OF 25 BIRDS
NO VACCINATION
1/4 Mile

FIG. 8.—FIELD STUDY OF FOWL TYPHOID

stages of avian typhoid. These wild birds feed on the infected ground as well as utilize the poultry food. The migration of these birds from farm to farm points toward the probability of their being a potent disseminator of this disease. Available literature does not record scientific data as to an outbreak of avian typhoid in the English sparrow, though the disease called canary typhoid, due to B. paratyphosus B. is recorded (12).

In order to obtain information as to the potential possibilities of the sparrow as conveyor of avian typhoid, a series of these birds were tested as to their susceptibility to this disease with the following results:

It was possible to infect and kill the English sparrow through subcutaneous inoculation and recover the organism from the liver and kidneys.

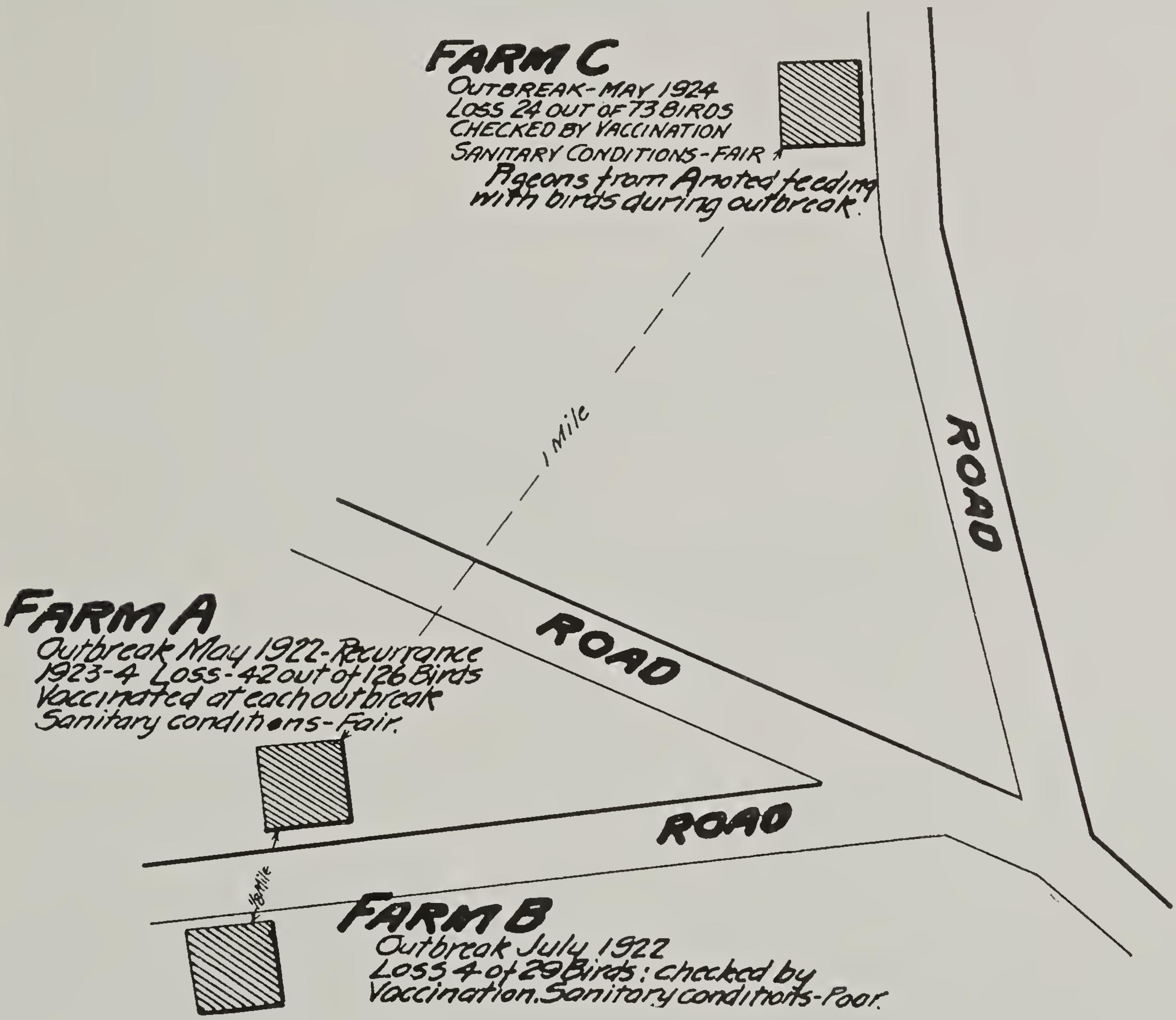
FARM C
OUTBREAK-MAY 1924
LOSS 24 OUT OF 73 BIRDS
CHECKED BY VACCINATION
SANITARY CONDITIONS-FAIR
Pigeons from A noted feeding with birds during outbreak.
1 Mile
ROAD
FARM A
Outbreak May 1922-Recurrance 1923-4 Loss-42 out of 126 Birds
Vaccinated at each outbreak
Sanitary conditions-Fair.
ROAD
ROAD
1/8 Mile
FARM B
Outbreak July 1922
Loss 4 of 29 Birds: checked by Vaccination. Sanitary conditions-Poor.

FIG. 9.—FIELD STUDY OF FOWL TYPHOID

No take could be secured through feeding the germ to these birds in the drinking water.

By sterilizing the feet of a sparrow with alcohol, and subsequently immersing the feet in bouillon cultures of avian typhoid, it was possible in two instances to recover E. sanguinaria 24 hours after.

Such evidence points toward the fact that the English sparrow is a possible conveyor of avian typhoid.

In two outbreaks investigated during the course of these studies the evidence pointed toward pigeons as the conveying agent. These outbreaks are graphically presented with explanatory data. Figures 8 and 9. The data as noted does not eliminate the sparrow or other mechanical agents of conveyance in these specific outbreaks, nor make light of the fact that in Figure 8 the dog was a possibility in the spread of the infection.

The common range ground is a common means of disseminating the disease in the flock and within flocks. Flocks ranging on the same grounds contract the disease through ingesting feed soiled by droppings of birds having the disease, or which are in the carrier stage. Such a condition probably caused the outbreak shown in Figure 10.

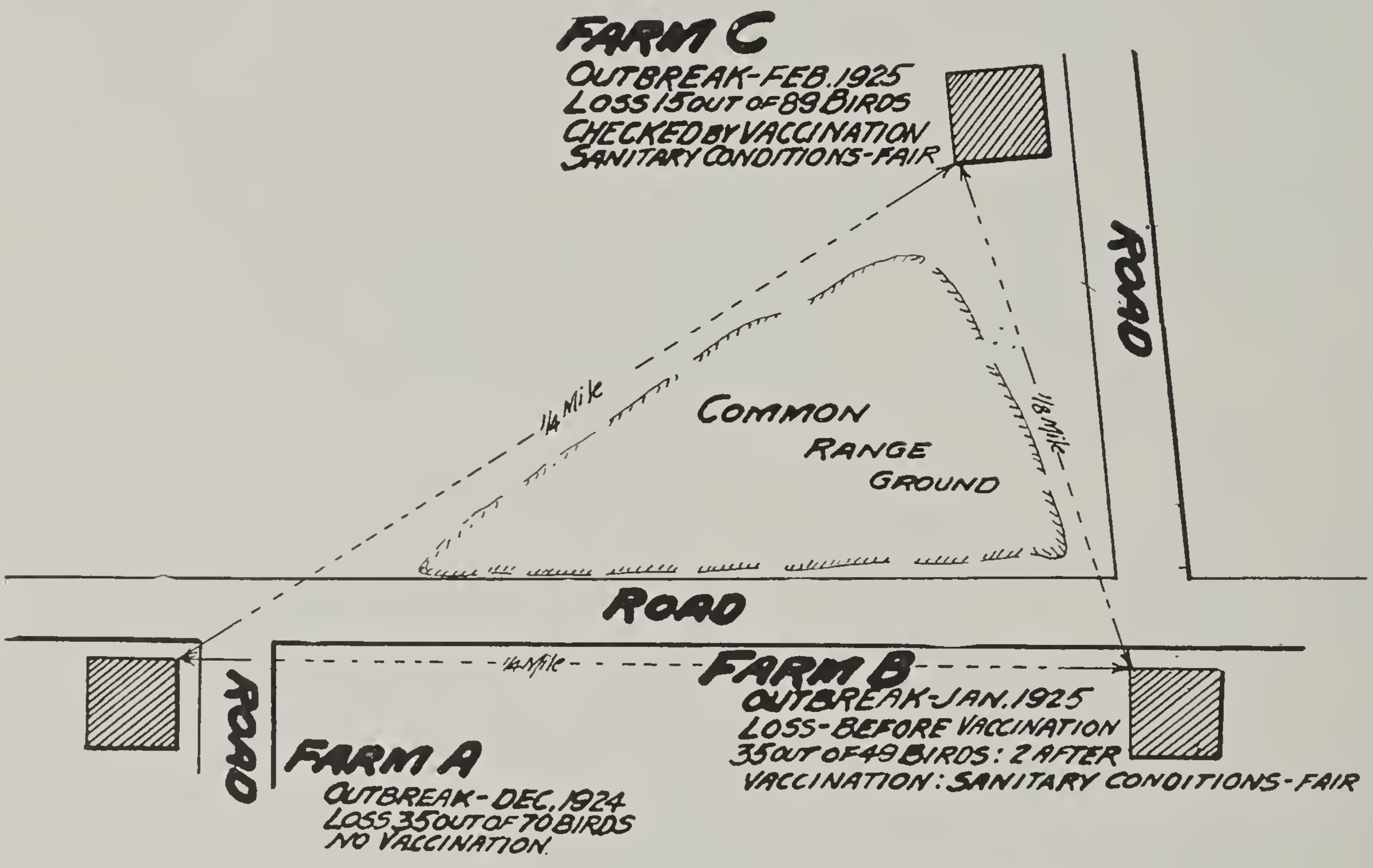
FARM C
OUTBREAK-FEB.1925
LOSS 15 OUT OF 89 BIRDS
CHECKED BY VACCINATION
SANITARY CONDITIONS-FAIR
ROAD
1/4 Mile
COMMON
RANGE
GROUND
1/8 Mile
ROAD
1/4 Mile
FARM B
OUTBREAK-JAN.1925
LOSS-BEFORE VACCINATION
35 OUT OF 49 BIRDS: 2 AFTER
VACCINATION: SANITARY CONDITIONS-FAIR
ROAD
FARM A
OUTBREAK-DEC.1924
LOSS 35 OUT OF 70 BIRDS
NO VACCINATION.

FIG. 10.—FIELD STUDY OF FOWL TYPHOID

Man and domestic animals, or wild animals, may also act as conveying agents of this and other avian diseases, though actual data on such means of transmission is difficult to obtain.

Other outbreaks investigated occurred with no traceable infecting agent. These occurred with an initial foci, and were featured by a progressive spread of the contagium, the flock outbreaks occurring at relatively regular intervals. Such outbreaks generally wear themselves out after covering a certain territory, but recurrence of the disease is probable in the infected area. Outbreaks with no traceable transmitting agent are shown in Figures 11 and 12.

The disease in North Carolina appears to be one of the late winter and early spring. Table III shows the date of inception as determined in the field investigations of this department:

Table III

Seasonal Occurrence of Fowl Typhoid in North Carolina

Month	*No. of Outbreaks*
January	5
February	10
March	9
April	11
May	10
June	0
July	1
August	1
September	1
October	3
November	2
December	3

Such findings concur with the observations of Truche (31) as to the seasonal occurrence of the disease in France, while teHennepe (33) reports the disease as most prevalent in the spring and early season in Holland.

An outbreak of the disease in the field usually has pronounced features. In North Carolina the disease seems to be restricted mostly to adults, although two outbreaks attacked half-grown chicks, and in one, young turkeys. Sometimes young birds fully feathered out are susceptible. Some of the outbreaks investigated from this department showed very high mortality among the adults, with no mortality occurring in the young chicks from the disease, though the young chicks ranged on the infected ground, and were subjected to such conditions as would ordinarily produce the disease in a susceptible

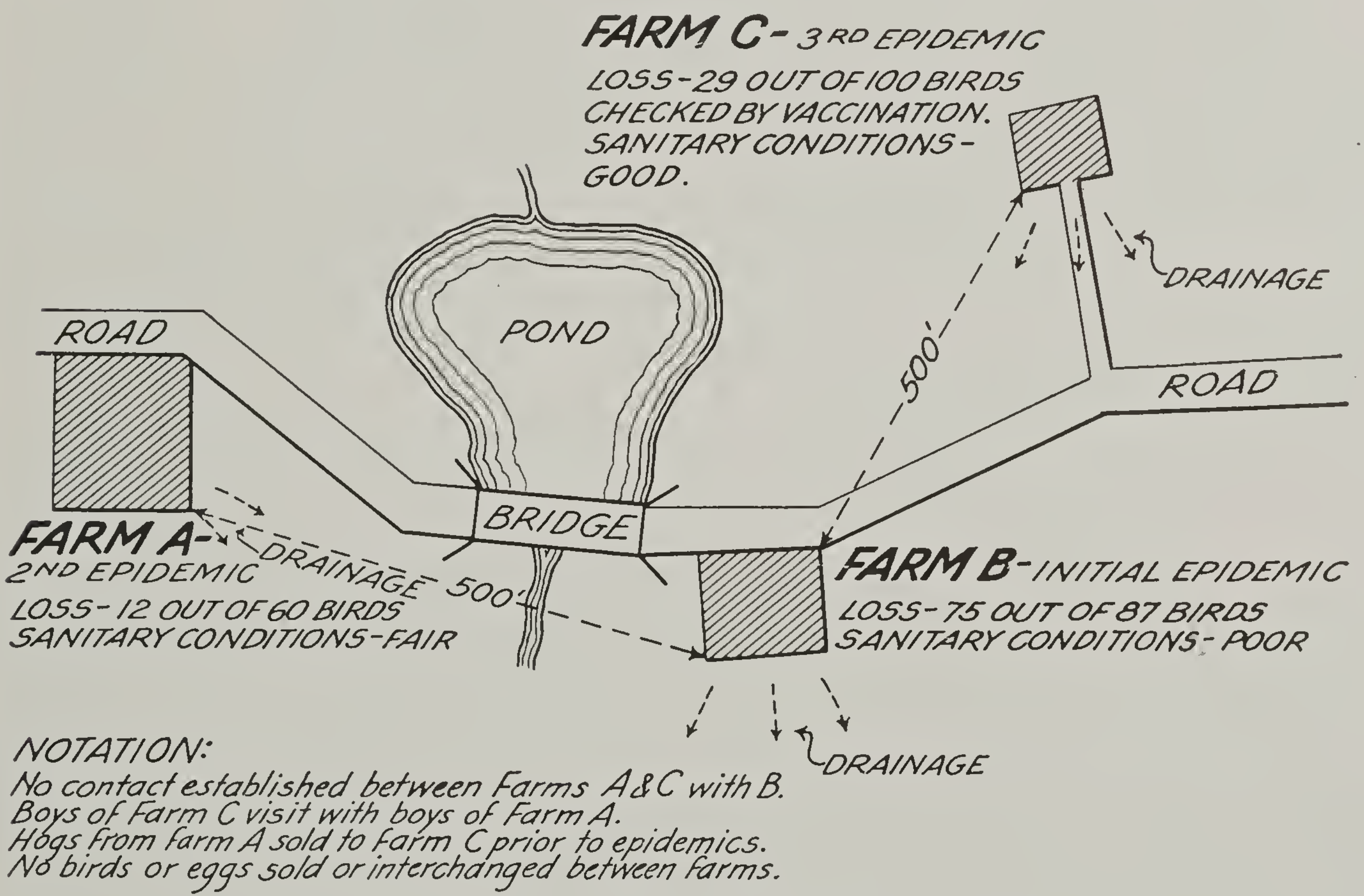
FARM C- 3RD EPIDEMIC
LOSS-29 OUT OF 100 BIRDS
CHECKED BY VACCINATION.
SANITARY CONDITIONS-GOOD.
DRAINAGE
POND
ROAD
ROAD
500'
BRIDGE
FARM A- 2ND EPIDEMIC
LOSS-12 OUT OF 60 BIRDS
SANITARY CONDITIONS-FAIR
DRAINAGE 500'
FARM B- INITIAL EPIDEMIC
LOSS-75 OUT OF 87 BIRDS
SANITARY CONDITIONS-POOR
DRAINAGE
NOTATION:
No contact established between Farms A&C with B.
Boys of Farm C visit with boys of Farm A.
Hogs from Farm A sold to Farm C prior to epidemics.
No birds or eggs sold or interchanged between farms.

FIG. 11.—FIELD STUDY OF FOWL TYPHOID

bird. This does not conform with the findings of Taylor (36) who found some mortality in the younger birds, but also reports high mortality in chicks, a large per cent of several batches dying in several days to several weeks of age, showing the same symptoms as adult birds, but not as well defined pathological changes being shown in post-mortem examination.

It is possible that chicks possess some natural immunity to the disease during the period of early infancy. Experimental work on the age of susceptibility will be presented later in this paper. Pigeons also seem to have some natural resistance to the disease under natural conditions, though in our artificial infection studies no difficulty was encountered in producing well-defined symptoms by infecting the bird per orum. Turkeys are mildly susceptible, although the susceptibility is much lower than that of the chicken. Guineas are slightly susceptible, but yield to artificial infection. Ducks and geese seem resistant in the field to avian typhoid, but show symptoms on being artificially infected.

A complete field study, together with clinical study of cases and anatomical findings of birds dying of the disease, is presented.

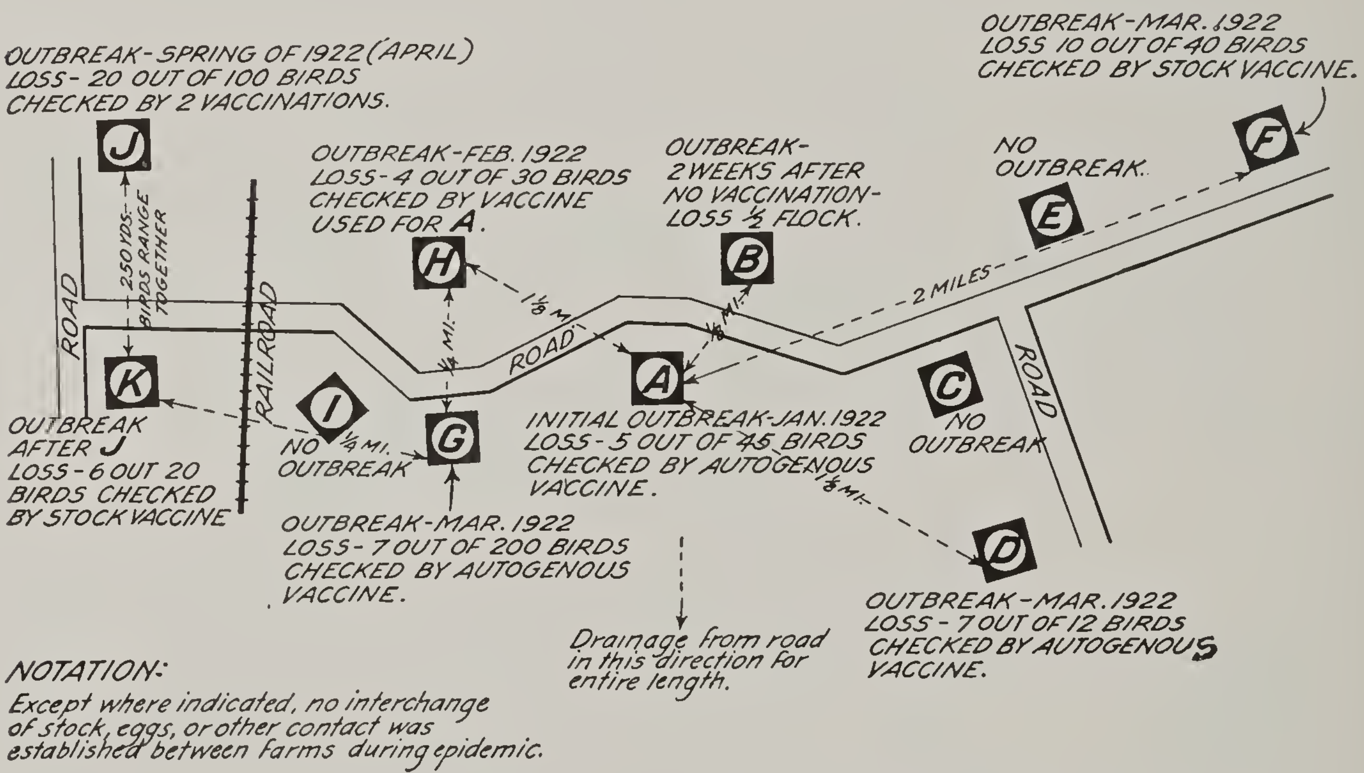
OUTBREAK-SPRING OF 1922 (APRIL)
LOSS- 20 OUT OF 100 BIRDS
CHECKED BY 2 VACCINATIONS.
J
250 YDS.- BIRDS RANGE TOGETHER
ROAD
K
OUTBREAK AFTER J
LOSS - 6 OUT 20 BIRDS CHECKED BY STOCK VACCINE
RAILROAD
I
NO OUTBREAK
¼ MI.
OUTBREAK-FEB. 1922
LOSS- 4 OUT OF 30 BIRDS
CHECKED BY VACCINE USED FOR A.
H
MI.
1⅛ MI.
ROAD
G
OUTBREAK-MAR. 1922
LOSS- 7 OUT OF 200 BIRDS
CHECKED BY AUTOGENOUS VACCINE.
OUTBREAK- 2 WEEKS AFTER NO VACCINATION- LOSS ½ FLOCK.
B
⅛ MI.
A
INITIAL OUTBREAK-JAN. 1922
LOSS - 5 OUT OF 45 BIRDS
CHECKED BY AUTOGENOUS VACCINE.
⅛ MI.
Drainage from road in this direction for entire length.
2 MILES
C
NO OUTBREAK
ROAD
D
OUTBREAK-MAR. 1922
LOSS - 7 OUT OF 12 BIRDS
CHECKED BY AUTOGENOUS VACCINE.
NO OUTBREAK.
E
F
OUTBREAK-MAR. 1922
LOSS 10 OUT OF 40 BIRDS
CHECKED BY STOCK VACCINE.
NOTATION:
Except where indicated, no interchange of stock, eggs, or other contact was established between farms during epidemic.

FIG. 12.—FIELD STUDY OF FOWL TYPHOID

FIELD STUDY OF OUTBREAK NUMBER TWO

This outbreak occurred in Wake County following the field studies shown in Figure 12. There was no traceable connection between these two outbreaks.

On July 3, 1922, a request was made to the Poultry Department by a plant having 900 adult birds. Sanitary conditions were excellent, the vitality of the flock high, and as noted above, no connection could be established between this flock and any flock suffering from typhoid infection. Eight of the range fowls were dead; two died the day of the investigation, and two sick ones were brought to the Experiment Station poultry hospital. Five of the eight dead birds were found in one of the houses, and the fact that examination of the carcasses revealed fully-grown maggots gave rise to the belief that all the birds had not died during the past night. The condition of the bodies showed that other birds in this respective pen had been feeding on them. From the above data, it was believed that the onset of the disease was about the 1st day of July.

Sick birds were identified by temperatures, the condition of the droppings, and the general behavior. The sick birds showed salivation, great prostration, difficulty and in some cases inability to walk, or if walking the gait was staggery; some of the birds were sitting with the ends of the beaks resting on the ground, and still others with the head and neck curved downward under the breast, which under ordinary conditions might have indicated limberneck. Post-mortem examination was made of the dead birds, the gross anatomical appearance being typical of avian typhoid, which indication was confirmed by laboratory cultures the next day.

On July 4th, 1 cc. of stock vaccine for avian typhoid was administered to all sick birds to observe the reaction. On July 5th an autogenous vaccine made from the organism isolated from the first dead birds was used, all birds on the farm being vaccinated. These included, in addition to the 900 chickens, 1 turkey, 11 waterfowls, and 12 pigeons. Young birds, including chicks in brooding, were vaccinated twice, the chicks receiving one-half dose.

It required three days from the initial onset for the disease to appear in an adjacent pen, and six days for it to appear in the second pen from the above, where it stopped, probably being checked by the vaccine. Thus it would appear that four days are required to produce immunity in the field against the disease. In other outbreaks, the disease appears to attack adult birds only, the disease being stopped at once, the vaccine also exerting a decided curative action on sick birds. In this outbreak only a few of the sick birds recovered as a result of the vaccine. In all, forty birds died.

Case Reports

CASE 1. JULY 4, 1922. A BUFF ORPINGTON COCKEREL, 5 MONTHS OLD

Clinical Study.—The bird was taken from the flock with a temperature of 112.5° F. Was thin in flesh. The respiration at 11 a. m. was 34, and at 4 p. m. 22. The bird stood with eyes closed, labored breathing, and gasping for air. Would not eat or drink. The droppings were of a sulphur color.

July 5, the temperature fell to 107.3°F. Condition much improved. Respiration 11 a. m. 34, 4 p. m. 26. Bird was able to walk and showed good appetite.

July 6, temperature 107.5° F.; respiration normal, appetite good.

July 7, temperature, respiration, and appetite normal. The bird was considered as recovered on the 8th, conditions being normal.

CASE 2. JULY 4, 1922. A BUFF ORPINGTON PULLET, 4 MONTHS OLD

Clinical Study.—The bird was taken from the flock at 11 a.m. with a temperature of 111° F.; respiration 36. The body of the bird was hot to the touch; droppings of a sulphur color; and poor in flesh. The face was pale or anemic. The bird did not eat or drink, and died at 4 p. m.

Gross Anatomy.—Rigor mortis has set in, though the bird has been dead only an hour; the liver and spleen are enlarged; heart congested and of a parboiled appearance. There is a small amount of edema in the region of the heart and liver. The kidneys are congested and in a state of cloudy swelling. The spleen is normal in size.

Cultures taken from the heart blood and from the liver show a luxuriant growth in 24 hours, the growth being identified as E. sanguinaria.

Microscopic Anatomy.—Specimens of the liver, heart, and kidneys were hardened in formalin, sectioned and stained in hematoxylin and eosin and clarified in beechwood creosote for study.

Liver.—Both active and passive congestion is present; areas are invaded by cells of inflammation, constituting acute hepatitis. Cloudy swelling exists, with some areas containing groups of cells loosing their nuclei, and nearing focal necrosis.

Heart.—Some areas show muscle fibers have lost their cross striation, and are apparently in a state of cloudy swelling. There is a general condition of congestion. The cloudy swelling causes a parboiled appearance on gross examination.

Kidneys.—There is intense congestion, both active and passive; cloudy swelling is present, some of the cells being nearly in a state of dissolution.

CASE 3. JULY 4, 1922. A BUFF ORPINGTON PULLET, 4 MONTHS OLD

Clinical Study.—The bird was extremely poor; temperature 113°F., respiration 26. Brought to the hospital; a condition of prostration existed, the bird sitting dozing with the head and tail resting on the floor of the coop, the eyes closed. Face anemic. The bird refused feed and water, and died at noon in convulsions.

Gross Anatomy.—Rigor mortis sets in very rapidly; the liver is in a state of inflammation and congestion, the blood dripping from the sectional surface. The heart is congested and presents a parboiled appearance. The kidneys are congested and in a state of cloudy swelling. There is a quantity of serous fluid around the heart and liver. The pericardial sac contains an increased quantity of serous fluid, a condition of hydropericarium. Cultures of the heart blood and liver proved positive for E. sanguinaria.

CASE 4. JULY 4. A BUFF ORPINGTON COCKEREL, 4 MONTHS OLD

Clinical Study.—The cockerel was taken from the flock at 10 a.m. with a temperature of 110.5° F., and a respiration of 54. The bird was poor in flesh, hot to the touch, and showed liquid, sulphur colored droppings. A condition of great prostration existed, and the bird refused feed and water. At 12:30 p. m. the respiration increased to 60, the bird lay on its side in a helpless state with the head curved forward under the body. The bird died at 2 p. m.

Gross Anatomy.—Rigor mortis set in very quickly after death; the carcass is emaciated; the heart is congested and has a parboiled appearance. There is a serous effusion about the heart and liver with an excess of fluid in the pericardium. The liver and spleen are enlarged and congested; the kidneys are congested and in a state of cloudy swelling.

Cultures from the heart and liver positive for E. sanguinaria.

CASE 5. JULY 4. S. C. RHODE ISLAND RED, 4 MONTHS OLD

Clinical Study.—This bird showed signs of illness at 10 a.m. It was noticed acting abnormally, isolated from the rest of the birds. The facial expression was one of sleepiness, with a slightly nervous twitching of the head. On approaching, the bird moved unsteadily, as though weak. In the hospital a temperature of 106.6° F., and respiration of 26 was noted. The bird was depressed and sat with the head and tail drooped. There was a watery, sulphur colored discharge from the bowel. The second day the temperature rose to 108.5° F., and the respiration to 44. The bird showed depression, and the body was hot to the touch. Saliva dribbled from the mouth. The third day showed a temperature of 109.5° F., the bird being in a condition of complete prostration. The bird died during the third day.

Gross Anatomy.—The liver is enlarged and friable. Blood drips from the sectioned surface. The heart is congested and in a state of cloudy swelling.

Cultures of the heart blood and liver proved positive for E. sanguinaria.

CASE 6. JULY 4. A PARTRIDGE PLYMOUTH ROCK PULLET, 5 MONTHS OLD

Clinical Study.—This bird was taken from the flock with a temperature of 110° F., and a respiration of 32. The bird was very much depressed, and sat with eyes closed and the head close to the floor. A sulphur colored discharge was emitted from the bowel. The second day showed a temperature of 109° F., and a respiration of 24, with the symptoms of the first day aggravated. The third day the temperature was 108.1° F. The bird was prostrated, lying on its side. Death occurred during the night. It was noticed that the temperature fell to practically normal before the death in this case, whereas the four previously described died at the height of the fever.

Gross Anatomy.—The liver is enlarged and congested; the heart is congested and of a parboiled appearance; the kidneys are congested and in a state of cloudy swelling.

Cultures of the heart blood and liver were made, the liver culture being positive and the heart negative for E. sanguinaria.

CASE 7. JULY 4. A BLACK SPANISH PULLET, 4 MONTHS OLD

Clinical Study.—Taken from the pen at 10 a.m. in a depressed and prostrate condition; face anemic; the body was hot to the touch, and there was a sulphur colored discharge from the bowel. The temperature was 112° F., and the respiration 28. The bird was given 2 cc. vaccine subcutaneously, and by 4 p. m. the temperature had dropped to 109° F. The bird stood with head and tail down, and with eyes closed. Feed and water refused. The second day the temperature had fallen to 108° F., and other symptoms subsided. Third day, the temperature and respiration were normal, the bird ate and drank normally, and by the fifth day showed no signs of the disease.

CASE 8. JULY 4. MOTTLED HOUDAN COCKEREL, 4 MONTHS OLD

Clinical Study.—Bird taken to poultry hospital with slight symptoms of avian typhoid. The temperature was 107.5° F., and respiration 30; in a fair condition of flesh. The bird stood most of the time with a depressed attitude. Took little feed or water. Vaccinated with 2 cc. of avian typhoid vaccine. On the second day the bird showed great improvement, which continued until the fifth day, when to all appearances the bird was normal.

CASE 9. JULY 8. RHODE ISLAND RED PULLET, 5 MONTHS OLD

Clinical Study.—Bird taken from the pen in the afternoon with a temperature of 108.5° F., and died during the night.

Gross Anatomy.—The liver is enlarged and friable; the heart is congested and presents a parboiled appearance.

Cultures of the heart blood and liver proved positive for E. sanguinaria.

CASE 10. JULY 8. RHODE ISLAND RED PULLET, 5 MONTHS OLD

Clinical Study.—The bird was removed from the pen in an advanced stage of the disease. The body was hot to the touch, and there was a sulphur colored liquid discharge from the bowel. The bird sat in a dozing condition, and when approached walked off showing a staggery gait. Temperature was 112° F., and respiration 36. The temperature on the second day was 109° F., and on the third 111° F. Respiration on the third day 36. The temperature on the fourth day was 110° F., and the bird died during the day.

Gross Anatomy.—The liver and spleen enlarged and congested; the heart is congested and presents a parboiled appearance; the kidneys are congested and in a state of cloudy swelling.

Cultures of the heart blood and liver, on nutrient agar, proved positive for E. sanguinaria.

CASE 11. JULY 8. RHODE ISLAND RED COCKEREL, 5 MONTHS OLD

Clinical Study.—The bird was taken from the pen with a subnormal temperature; the vitality was low, and the bird walked with a weak, staggery gait. During the night the temperature increased to 109° F., with a respiration of 36. The second day showed no change of respiration and temperature, and the bird died during the day. This bird had been vaccinated four days prior while on range.

Gross Anatomy.—The liver is enlarged and friable; the heart is congested and pale; the kidneys are congested and in a state of cloudy swelling.

Cultures from the heart blood and liver proved positive for E. sanguinaria.

CASE 12. JULY 8. A BUFF ORPINGTON PULLET, 5 MONTHS OLD

Clinical Study.—This pullet had received one vaccination before being taken from the pen, and was given another vaccination. The temperature of the bird was 112° F., face anemic. The bird died during the night.

Gross Anatomy.—The liver is enlarged and congested; the heart is congested and pale; the kidneys are in a state of congestion and cloudy swelling.

Cultures from the heart blood and liver proved positive for E. sanguinaria.

CASE 13. JULY 8. A BARRED PLYMOUTH ROCK PULLET, 4 MONTHS OLD

Clinical Study.—This bird had previously been vaccinated while a member of the flock. The temperature on removal from the flock was 110.5° F., and respiration 26. The bird was poor in flesh, face anemic. The temperature rose to 111° F. on the second day, and dropped to 110° F. on the third day, when the bird died.

Gross Anatomy.—The liver and spleen are enlarged and congested; the heart is congested and pale; the kidneys are congested and in a state of cloudy swelling.

Cultures from the heart blood and liver proved positive for E. sanguinaria.

CASE 14. JULY 8. RHODE ISLAND RED PULLET, 5 MONTHS OLD

Clinical Study.—The bird was isolated from the flock with typical symptoms of the disease, showing a temperature of 111° F. This bird had been vaccinated July 4th, and was given 2 cc. more vaccine. The bird died during the night.

Gross Anatomy.—The heart, spleen, liver, and kidneys showed similar conditions to those above.

Cultural tests of the heart blood and liver proved positive for E. sanguinaria.

CASE 15. JULY 8. S. C. R. I. RED PULLET, 4 MONTHS OLD

Clinical Study.—This bird was in poor condition, partially prostrated, had lost appetite and refused to drink. Had been vaccinated on July 4th, was taken to poultry hospital and revaccinated with 2 cc. of avian typhoid vaccine. The temperature was 109° F. On the second day the temperature was 110° F., and respiration 26. The third day the temperature was 109° F. and the respiration 32. To all appearances the bird was improving, but it died the following morning.

Gross Anatomy.—The gross anatomy was similar to that of the previous cases which had died of the disease.

Cultural tests of the heart blood and liver proved positive for E. sanguinaria.

CASE 16. JULY 8. S. C. RHODE ISLAND RED PULLET, 4 MONTHS OLD

Clinical Study.—This bird was removed from the flock at the same time as Cases 14 and 15, and showed similar symptoms. The temperature on removal was 110.1° F. The bird died during the night.

Gross Anatomy.—The heart, liver, spleen, and kidneys showed a similar condition to the previous cases which had died of the disease.

Cultures from the heart blood and liver proved positive for E. sanguinaria.

CASE 17. JULY 8. S. C. RHODE ISLAND RED PULLET, AGE 4 MONTHS

Clinical Study.—This bird was removed from the flock at 1 p.m., July 8th, poor in flesh, and very weak. Anemic face. There was a sulphur colored discharge from the bowel. Injection was made with avian typhoid vaccine, the bird having been vaccinated July 4th. The temperature was 108° F., and respiration 32. The temperature on the second day and third day was 108° F., but the respiration had risen to 42. On the fourth day the temperature increased to 110.5° F., and the respiration dropped to 22. The fifth day the temperature dropped to 108° F., and the respiration was 24; the bird moved about to some extent, ate and drank. The bird died during the night.

Gross Anatomy.—The gross anatomy was typical of previous cases dying of the disease.

Cultural tests of the heart blood and liver were positive for E. sanguinaria.

CASE 18. JULY 8. S. C. R. I. RED PULLET, 5 MONTHS OLD

Clinical Study.—This bird had been vaccinated on July 4th. The symptoms were similar to the preceding ones. The bird was taken to the poultry hospital and revaccinated. The temperature was 110.2° F. The bird died during the night.

Gross Anatomy.—The heart, liver, spleen, and kidneys showed the same gross anatomical conditions as the preceding cases.

Cultural test of the heart blood and liver proved positive for E. sanguinaria.

CASE 19. JULY 8. S. C. R. I. RED COCKEREL, 5 MONTHS OLD

Clinical Study.—The bird was poor in flesh, weak, and walked with a staggery gait. The temperature was 110° F., and the respiration 22. The bird had been vaccinated on July 4th, and was revaccinated on being brought to hospital. The second day the bird had a temperature of 108° F. in the morning, which temperature rose to 112 by evening, with a respiration of 26. The bird seemed stupid and refused feed and water. The third day the temperature was 110.5° F., and the respiration 24. A condition of prostration existed, water and feed being refused, death occurring during the night.

Gross Anatomy.—An abdominal hemorrhage is present; the liver is congested and friable, blood dripping from the sectional surface. The heart is congested and presents a parboiled appearance. The kidneys are congested and in a state of cloudy swelling.

Cultural tests from the heart blood and liver proved positive for E. sanguinaria.

CASE 20. JULY 10. A PARTRIDGE PLYMOUTH ROCK PULLET, 3 MONTHS OLD

Clinical Study.—The bird was taken from the pen with a temperature of 109° F., and a respiration of 30. The second day the temperature was 108° F., with the respiration 32. The temperature rose to 109.2° F., with a respiration of 30 by the second afternoon. The third day the temperature was 109° F. and respiration 28. The fourth day the temperature fell to 108° F. and respiration was 28. From this time on the bird grew stronger and threw off the disease.

CASE 21. JULY 10. A BUFF ORPINGTON PULLET, 5 MONTHS OLD

Clinical Study.—The bird was taken from the flock at 10 a.m. with a temperature of 108° F., and a respiration of 26, pale of face. Apparently the disease was in its onset, and the bird was revaccinated, the first vaccination being administered on the 4th. By 4 p. m. the temperature fell to 107.5° F., but rose to 108° F. the following morning. The bird was slightly drowsy, this being most pronounced when the bird was left alone and not under any degree of excitement. The third day showed a temperature of 107.3° F., the bird dying during that night.

Gross Anatomy.—An abdominal hemorrhage is present. The liver is enlarged and friable. The heart is congested and parboiled in appearance. The kidneys are congested and in a state of cloudy swelling.

Cultural tests from the heart blood and liver prove positive for E. sanguinaria.

CASE 22. JULY 10. A RHODE ISLAND RED PULLET, 4 MONTHS OLD

Clinical Study.—The bird was poor in flesh, showed a temperature of 111° F., and a respiration of 48. A condition of prostration existed, the bird being without the use of the neck muscles, giving a clinical picture of limberneck. Death occurred at noon.

Gross Anatomy.—Rigor mortis set in quickly. The bird is in a high degree of emaciation. The liver and spleen are congested; the heart is congested and pale. The kidneys are congested and in a state of cloudy swelling.

Cultures from the heart blood and liver prove positive for E. sanguinaria.

CASE 23. JULY 10. A S. C. WHITE LEGHORN PULLET, 4 MONTHS OLD

Clinical Study.—Bird removed from the flock with a temperature of 109° F., and a respiration of 24. In a condition of stupor, the head down and eyes closed. Taken to poultry hospital and revaccinated. At 4 p. m.

the temperature had fallen to 108° F. The second morning showed a temperature of 110° F., and a respiration of 43. The bird was gradually becoming worse, and died in the afternoon, in convulsions.

Gross Anatomy.—The carcass is poor; there is edema in the region of the heart and liver. The liver is congested, enlarged, friable, and the blood drips from the sectioned surface. The heart is congested and presents a parboiled appearance. The kidneys are congested and in a state of cloudy swelling.

Cultures from the heart blood and liver prove positive for E. sanguinaria.

CASE 24. JULY 10. A S. C. RHODE ISLAND RED COCKEREL, 4 MONTHS OLD

Clinical Study.—Isolated from flock at 10 a.m., July 10th, with temperature of 108° F., and a respiration of 28. The bird was depressed and drowsy, and would not take feed or drink. Died in the evening.

Gross Anatomy.—There is edema about the heart and thoracic region. Hepatitis is present, and blood drips from the sectioned surface of the liver. There is hemorrhage in the abdominal cavity. The heart is congested and pale in color. The kidneys show congestion.

Cultures from the heart blood and liver prove positive for E. sanguinaria.

CASE 25. JULY 10. A. S. C. RHODE ISLAND RED PULLET, 4 MONTHS OLD

Clinical Study.—Brought to the poultry hospital at 9 a.m. on the 10th, showing initial symptoms of fowl typhoid. The temperature was 108° F., and respiration 26. At 4 p. m. the temperature had risen to 108.5° F. and respiration 30. The bird was still on its feet, would move, ate and drank. The temperature on the second day, in the forenoon, was 108.5° F., but by night it had risen to 109° F., with a respiration of 36. The bird's appetite remained good. The temperature on the morning of the third day dropped to 107.5° F., but rose to 108.5° F. in the afternoon. The fourth day the temperature dropped to 107.5° F., from which time the bird appeared to throw off the disease, recovery taking place.

CASE 26. JULY 10. A S. C. R. I. R. PULLET, 4 MONTHS OLD

Clinical Study.—Bird was taken from the flock with a temperature of 109.1° F., and a respiration of 28. Was in a fair state of flesh, able to walk, eat and drink; face anemic. This bird received a second dose of vaccine, the first being administered on the 4th. The temperature fell to 108.1° F. in the afternoon. The temperature on the morning of the second day was 109° F.. and was 109.4° F. in the afternoon. The respiration was 26. The afternoon of the third day showed that the temperature had fallen to 108.5° F., and the bird appeared to be regaining its vitality. From this time on the bird gained, and later made full recovery.

CASE 27. JULY 10. S. C. R. I. RED COCKEREL, 4 MONTHS OLD

Clinical Study.—Brought to poultry hospital at 9 a.m. with a temperature of 109° F. The bird died at 3 p. m.

Gross Anatomy.—The liver, spleen, heart, and kidneys showed similar gross conditions as noted before in birds succumbing to this disease.

CASE 28. JULY 10. A S. C. R. I. RED PULLET, 4 MONTHS OLD

Clinical Study.—Brought to hospital at 9 a.m. with a temperature of 109° F. By 4 p. m. the temperature had increased to 110.5° F., with a respiration of 24. The bird appeared stupid and depressed. The second morning showed a temperature of 109° F. and a respiration of 34. By 4 p. m. of the second day the temperature had fallen to 108° F. The temperature on the third morning had increased to 109° F., and was 110° F. by 4 p. m. The bird was revaccinated. From this time on the temperature fell to normal and the bird threw off the disease.

CASE 29. JULY 10. S. C. R. I. RED PULLET, 4 MONTHS OLD

Clinical Study.—Isolated from flock at 9 a.m. with a temperature of 109° F. The bird was depressed. By 4 p. m. the temperature had risen to 110.5° F. The second morning the temperature had fallen to 109.5° F., but the bird's physical condition became worse, and it died during the day.

Gross Anatomy.—The heart, liver, spleen, and kidneys showed the usual anatomical features typical of the disease.

Cultures of the heart blood and liver proved positive for E. sanguinaria.

CASE 30. JULY 10. A. S. C. R. I. RED PULLET, 4 MONTHS OLD

Clinical Study.—Bird taken to poultry hospital at 9 a.m. with a temperature of 110° F. Was revaccinated, the first vaccination having been administered on the 4th. By 4 p. m. the temperature had increased to 111° F. The morning of the second day showed a temperature of 110° F., and the afternoon 108° F. This temperature was held during the morning of the third day, but rose to 109.8° F. by afternoon. The temperature from this time on fell steadily to normal, and the bird recovered.

CASE 31. JULY 10. A BUFF ORPINGTON COCKEREL, 5 MONTHS OLD

Clinical Study.—Isolated from the flock at 1 p.m. with a temperature of 108° F. and a respiration of 24. This bird was apparently in the first stages of the disease. The bird was revaccinated. The temperature on the second morning was 108.5° F., and increased to 109° F. by afternoon. The morning of the third day showed a temperature of 110.5° F., but it was reduced to 109° F. by afternoon. From this time on, improvement was rapid, and the bird made recovery.

CASE 32. JULY 10. A BUFF ORPINGTON PULLET, 4 MONTHS OLD

Clinical Study.—Brought to the hospital with a temperature of 110° F. and a respiration of 36. The bird died during the night.

Gross Anatomy.—The liver is enlarged and congested; the kidneys congested and in a state of cloudy swelling; the heart is congested and presents a parboiled appearance.

Cultures taken from the heart blood and liver proved positive for E. sanguinaria.

CASE 33. JULY 10, 1924. A BUFF ORPINGTON PULLET, 4 MONTHS OLD

Clinical Study.—Brought to hospital at 1 p.m. with a temperature of 110° F., and a respiration of 32. The second morning showed a temperature of 110° F., but by 4 p. m. the temperature had risen to 111° F. The temperature on the third morning was 110.5° F., the fourth morning 111° F., and 110° F. in the afternoon. The bird was prostrate, hot to the touch, and refused feed and water. The bird died during the fifth day.

Gross Anatomy.—Hemorrhage is present in the abdomen; edema in the chest and region of the heart. The pericardial fluid is increased in quantity. The heart presents small, whitish, tumor-like masses, and has a parboiled

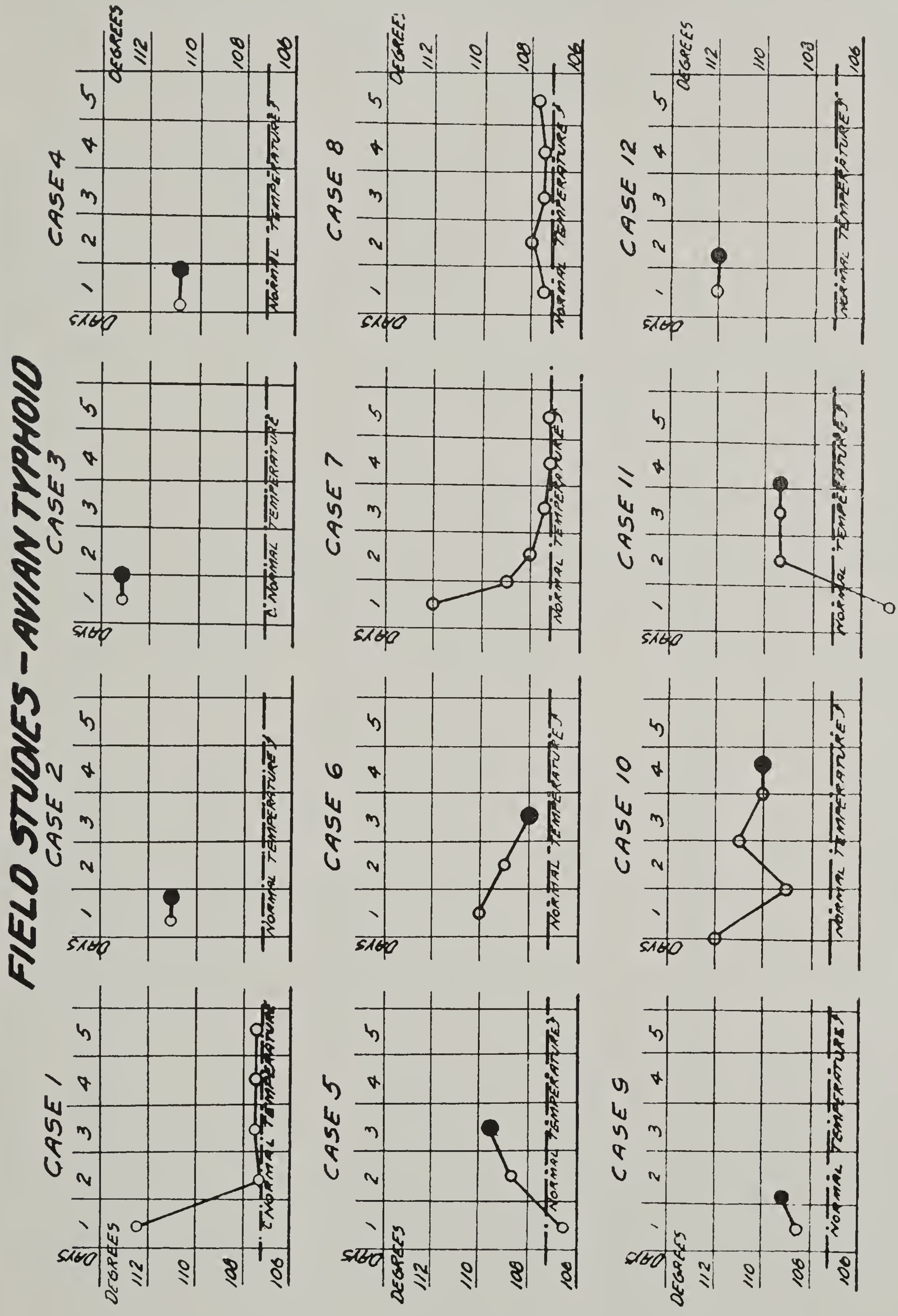

FIG. 13.—HOSPITAL TEMPERATURE CHARTS OF FIELD CASES OF AVIAN TYPHOID

appearance. The liver is enlarged, congested, and blood drips from the sectioned surface. The kidneys are congested and in a state of cloudy swelling.

Cultural tests of the heart blood and liver are positive for E. sanguinaria.

Microscopic Anatomy.—Liver, both active and passive congestion are present. Cloudy swelling is present.

Kidneys, active and passive congestion are present. Cloudy swelling exists.

Heart. The heart is congested. Areas of acute inflammation are present.

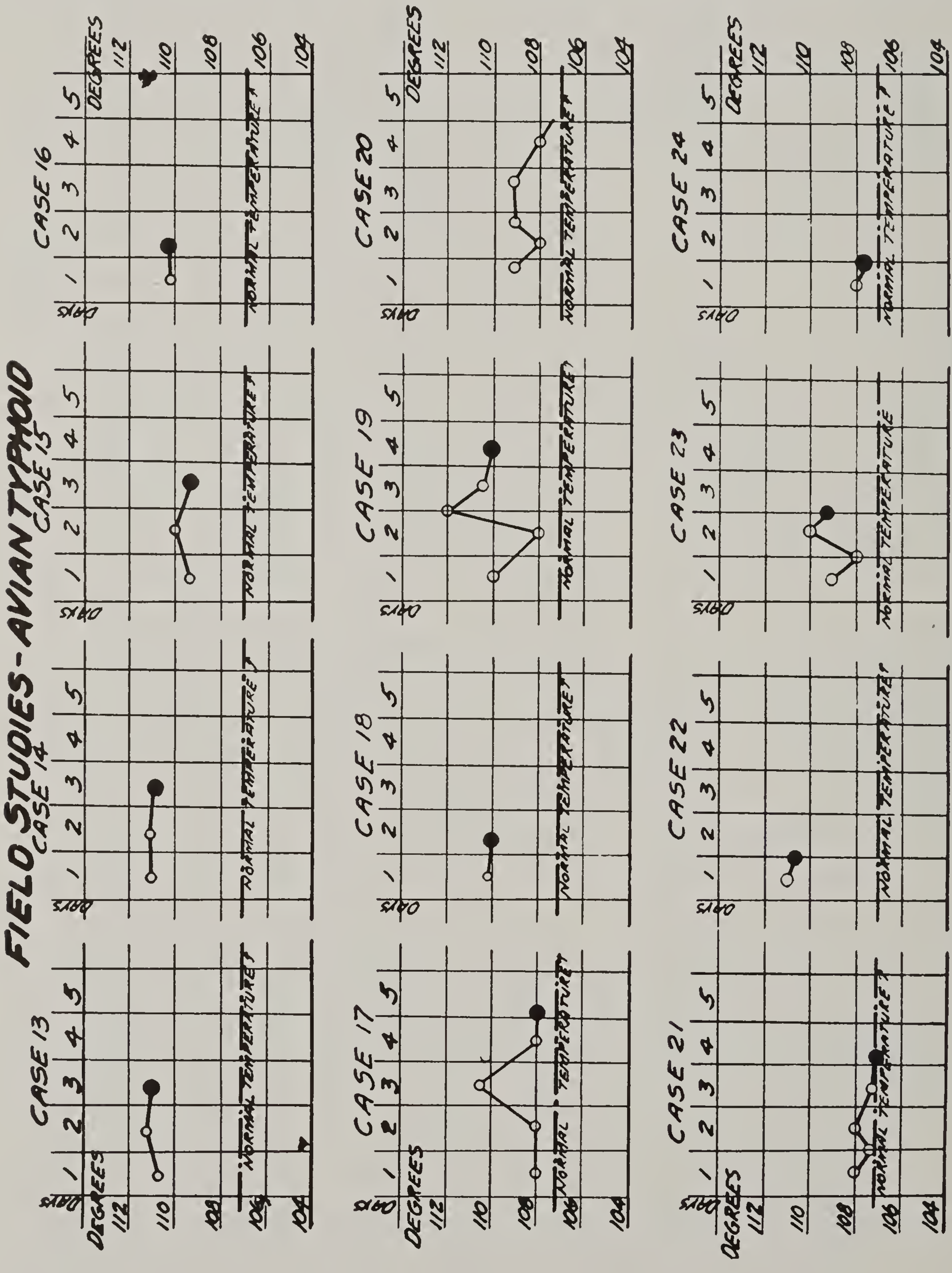

FIG. 14.—HOSPITAL TEMPERATURE CHARTS OF FIELD CASES OF AVIAN TYPHOID

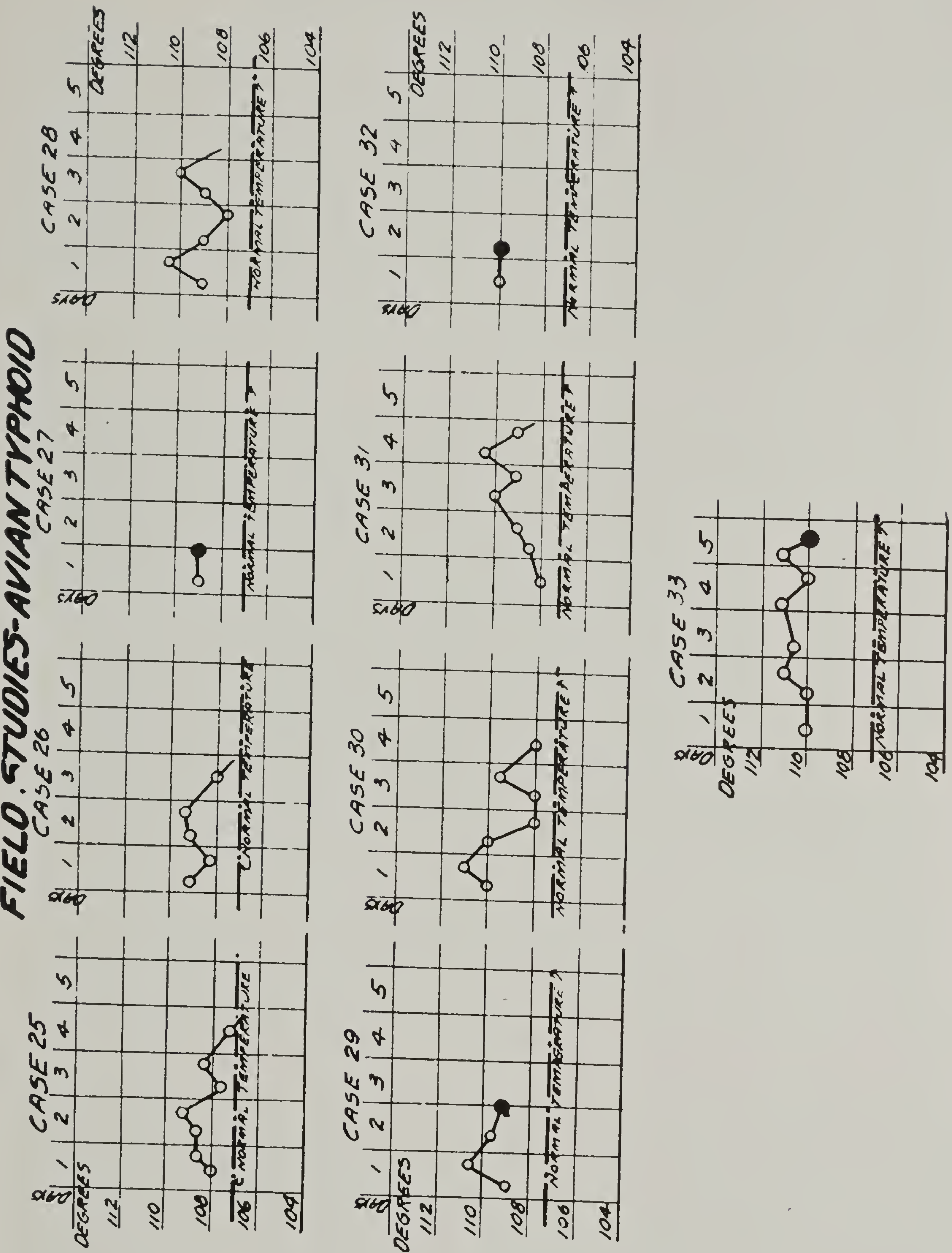

FIG. 15.—HOSPITAL TEMPERATURE CHARTS OF FIELD CASES OF AVIAN TYPHOID

DISCUSSION

This outbreak of avian typhoid is typical of the disease, occurring without warning, and with no traceable cause. The foci of infection was one of the range pens, where the disease was first recognized, and which sustained the highest mortality. The disease spread to two adjacent pens, the topography of the ground being favorable for such spread.

The mortality in this outbreak was 40 birds, or 4.4 per cent. Prompt administration of vaccine, isolation and destruction of all cases, and strict sanitary precautions checked the disease. The outstanding feature of this outbreak was the fact that the disease broke out among young range birds

4 to 5 months of age. Of the 33 hospital cases studied, 24 or 72.7 per cent died, and 9 or 27.3 per cent resisted the disease. It is probable that all of these birds had ingested the germ before the vaccine administered could become effective, and body immunity against the disease be built up. The eight cases isolated July 4th had such well-defined symptoms of the disease that vaccination represented only an experimental procedure. Positive conclusions as to the efficacy of vaccination of birds showing symptoms of the disease cannot be definitely arrived at, although it is quite possible that some beneficial effect is derived from same. From a practical standpoint, the vaccination of sick birds is to be strongly advised against, such birds should be destroyed because of the fact that they might become carriers.

Two vaccinations were given all birds, including range chicks.

Figures 13, 14, and 15 are hospital temperature charts showing fluctuation of the body temperatures of 33 birds with fowl typhoid. Of the 33 hospital cases, 9 or 27 per cent recovered. The temperature may be erratic, that is, up one day with a drop on the following day, only to rise again on the succeeding day. The temperature varies according to the severity of the disease, rising from a normal temperature of 107.3° F. to as high as 113°F. In this outbreak, the course of the disease varied, some birds dying the same day or the following day of the initial symptoms, while others would live four or even five days and then die, or the sub-acute cases recover after a lapse of four or five days.

SYMPTOMS OF THE DISEASE

The symptoms observed in this outbreak among the young birds are as follows:

In the initial stages, the symptoms may be overlooked if the birds are in any way alarmed. Unalarmed, the birds may show signs of dozing. When awakened, especially if startled to a small degree, the bird does not present a normal facial appearance, and the action of the head is of a nervous type. The bird on arising will show symptoms of weakness of gait. These symptoms are progressive until the bird becomes prostrate. In the advanced stages, the bird when at ease for a moment will sit with the head and tail down (Figure 16), and when it moves shows signs of exhaustion. Loss of flesh may be rapid, according to the severity of the disease. The bird may become partially comatosed, and if sitting, the head may fall forward till the beak touches the ground, or in a complete state of coma, when the bird lies on its side with the head curved under its breast. At this stage there is complete loss of the use of the muscles of the neck, and the bird is unable to walk. The bird may die without a struggle, or it may die in spasms. There is soon observed an unkempt appearance of the feathers, and a sulphur colored liquid discharge from the bowel. The face, comb, and wattles usually are highly anemic.

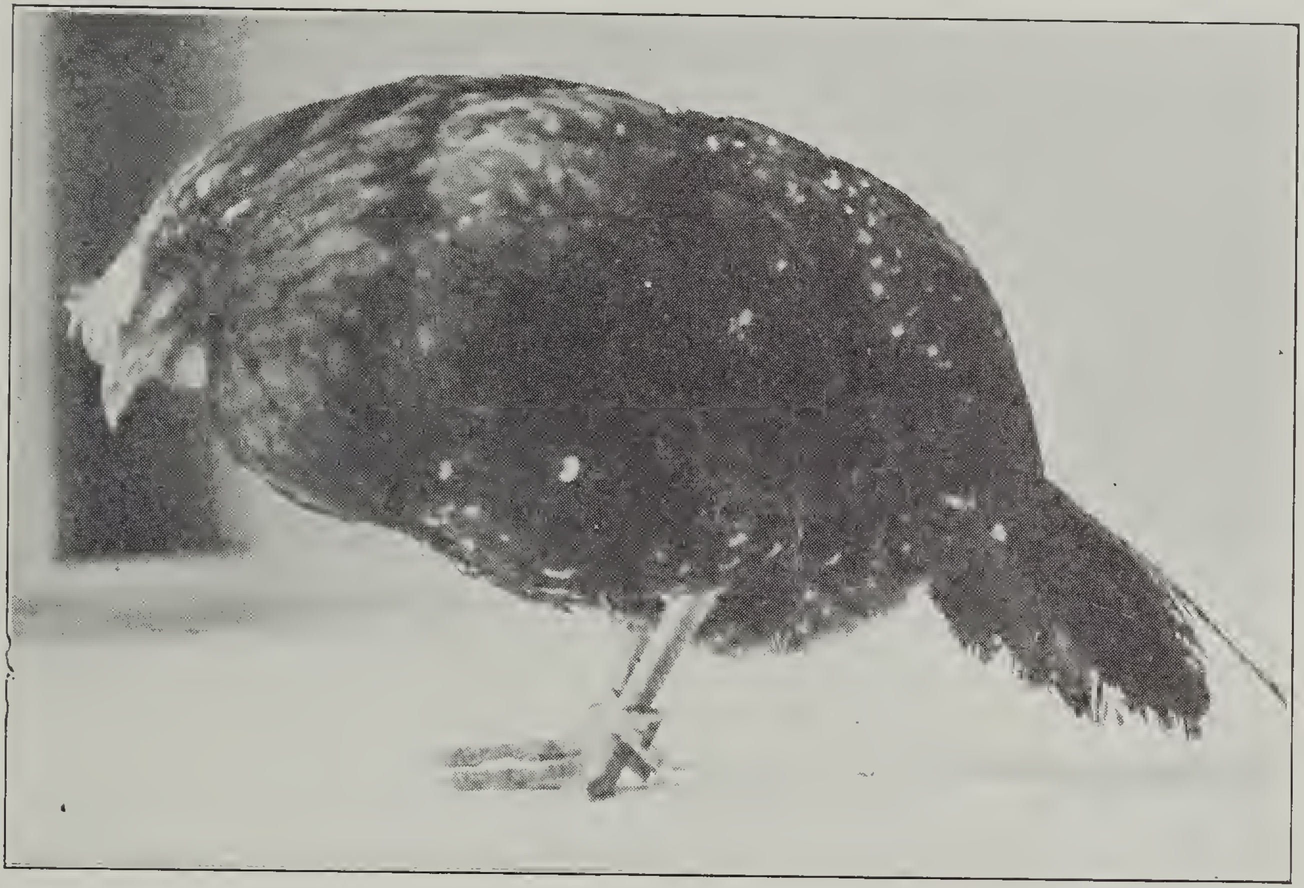

FIG. 16.—PHOTOGRAPH OF MOTTLED ANCONA HEN SHOWING TYPICAL SYMPTOMS OF FOWL TYPHOID

GROSS PATHOLOGY

The carcass is usually emaciated, the degree of emaciation depending on the severity and course of the disease. The face, comb, and wattles are anemic; edema may be present in the thorax, or may extend over the liver. The pericardeal fluid may be increased in quantity. More rarely there may be abdominal hemorrhage with suggilations of the mucous membrane lining the intestines.

The liver is usually enlarged (Figure 17), friable, sometimes streaked, and blood drips from the sectioned surface; the spleen is usually enlarged; the kidneys are congested and in a condition of cloudy swelling. The heart is usually congested. The lungs are usually normal, though in acute cases may be in a serous condition.

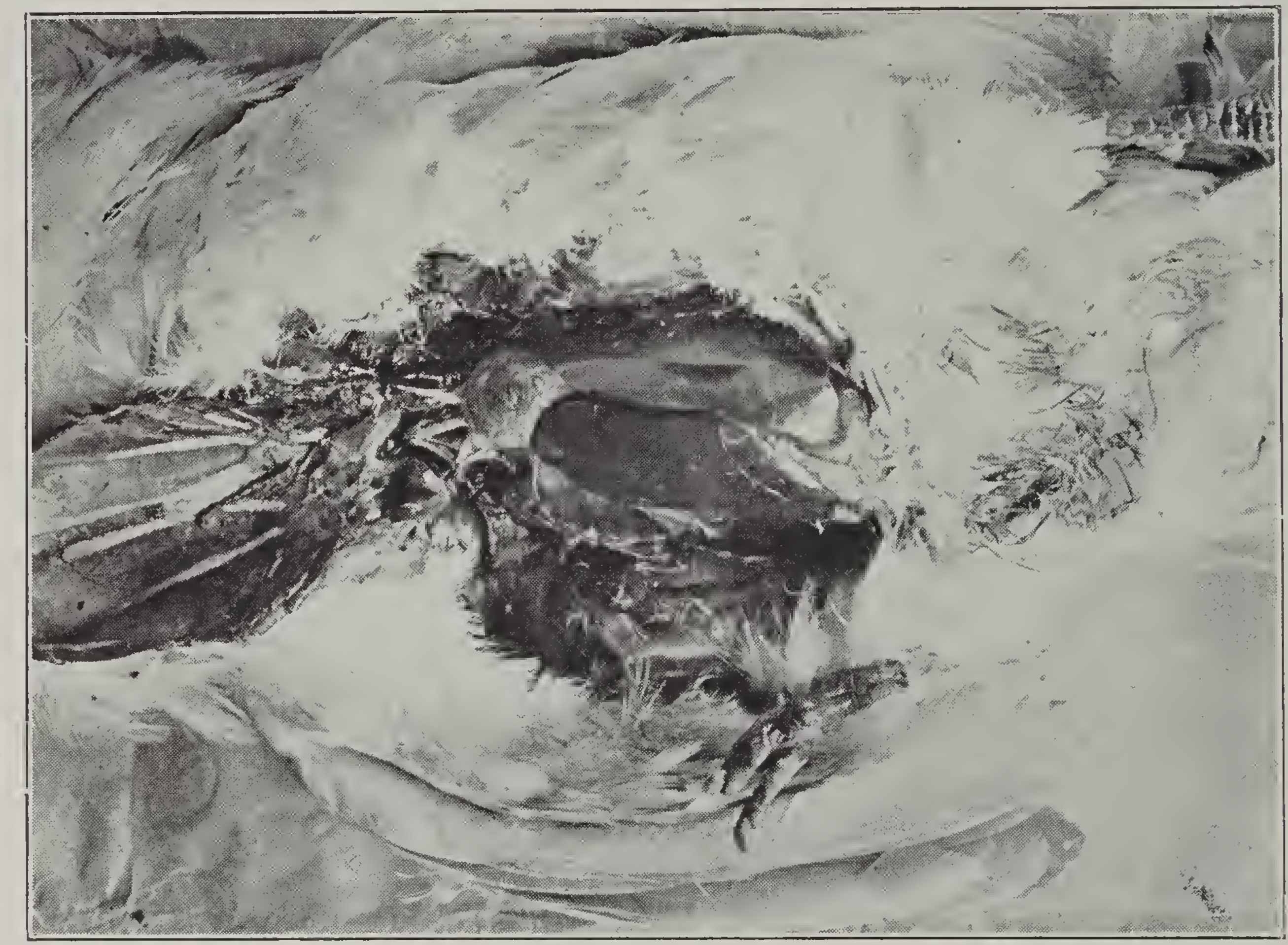

FIG. 17.—GROSS APPEARANCE OF LIVER IN RELATION TO ABDOMINAL CAVITY IN FOWL TYPHOID

MICROSCOPIC PATHOLOGY

Upon preparation of tissues, from various organs of the body, and a study under the microscope, profound changes are observed, especially in the parenchymatous organs.

The Kidneys.—The Eberthella sanguinaria lives and multiplies in the blood where it gives off a toxin. The toxin is probably first liberated through the arterioles; as a result active congestion, most marked in the cortical portions, is usually present in the kidneys. A weak heart and a damming back of the blood accounts for passive congestion which may be present, especially well marked in the veins of the medullary portion of the kidney. A closer examination under a higher magnification shows the greater injury to the cortical portions of the kidney, and especially to the cells of the convoluted tubules. This can be accounted for by the fact that these cells are the ones that eliminate the toxins, consequently getting more poison. In some areas the cells are in a state of cloudy swelling caused by the toxins from the E. sanguinaria, and are swollen because they have absorbed fluid. The absorption of water causes a precipitation of the proteids with a result that the cells become cloudy, hence cloudy swelling, or albuminoid degeneration. The nuclei and cell cytoplasm of the cells of some areas are undergoing advanced digestion, the nuclei disappearing, the cells leaving their base, pushing in, partially or completely obliterating the lumen, finally disintegrating, indicating a center of focal necrosis. Areas are invaded by leucocytes indicating infection or concentration of the toxin at this point and constituting inflammation or nephritis. As a summary, it may be said that acute parenchymatous nephritis is present.

The Liver.—An examination under a lower magnification, of a prepared section of the liver, shows active congestion which is most marked at the arterioles of the periphery of the liver lobule. Passive congestion, as affecting the central vein and its tributaries, is present and is caused by a damming back of the venous blood. An examination under a higher magnification shows the hepatic cells to appear swollen because they have taken up fluids. This absorbed water causes a coagulation of the cell proteids, hence a swollen, granular appearance of the cell, which may make the nucleus less distinct, is present. In some areas a digestion of the nuclei, and to some extent the cytoplasm, has caused a disintegration of the cells, and consequently a point of beginning necrosis. These conditions are brought about by the toxins of the E. sanguinaria. Blood reaching the periphery of the lobule first would give to this part the most toxins, with a result that these focal centers of necrosis are, for the most part, located in the periphery of the lobules of the liver. Areas are invaded by leucocytes due to the concentration of toxins or foci of infection of the E. sanguinaria. In these areas (see Figure 26) may be seen cells in a state of cloudy swelling or necrosis, leucocytes, other cells probably epithelioid replacing the hepatic cells and congestion of the blood vessels—both arteries and veins.

As a summary, it may be said that acute parenchymatous hepatitis is present.

The Spleen.—A microscopic study of prepared sections of the spleen shows similar conditions to those described in the liver, including both active and passive congestion. Acute spleenitis, then, may be present.

The Heart.—Congestion may be present. In some cases the muscle fibers have partially or completely lost their cross striation.

In conclusion we may say as Delafield and Prudden (5) say of human typhoid, so in fowl typhoid, toxemia may be manifested by disturbances in the circulatory, respiratory, and heat regulating mechanism, and in general metabolism, as well as by manifest lesions, such as albuminous (cloudy swelling) or other degenerations of parenchyma cells throughout the body, and alterations leading to leucocytosis.

The spread of the disease in a flock is very rapid, and is usually recognized by the owner only after a number of birds have died. The symptoms on the onset are undecided, and not likely to be recognized by the casual observer. The affected birds become dull, droopy, and tend to segregate themselves from the rest of the flock. There is a sulphur colored discharge from the bowel, an elevation of temperature, and usually the birds become acutely thirsty, but lack appetite. As the disease advances, the symptoms become more marked, and mortality ensues. There is a dribbling of saliva from the mouth; many birds become too weak to walk; others walk with a staggery gait; some sit with the heads resting on the ground, eyes closed, and in a condition of partial coma. The temperature may rise to 113 degrees, the normal temperature being about 107.5 degrees. Sometimes the disease is confused by the owner with limberneck. Before death, complete prostration usually occurs, the diarrhea being profuse and watery, and the bird becomes more or less anemic according to the duration of the disease. Prior to death, there is usually a well-defined drop in temperature. Occasionally the birds die in convulsions. The disease may assume a very acute form, with a quick death; it may present the normal features of the disease, with death and recovery as the termination, or it may assume a long drawn out form which

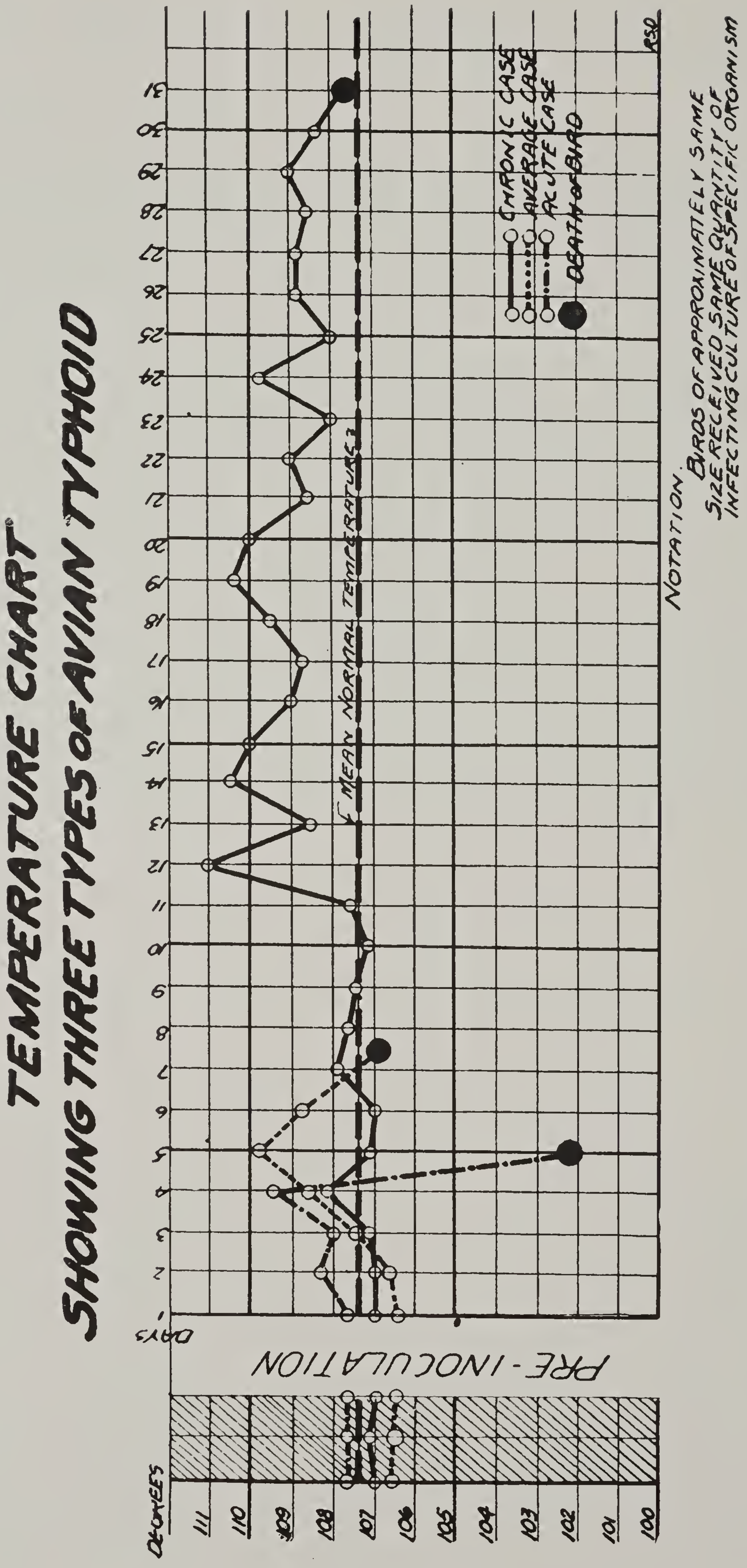

FIG. 18.—COMPOSITE TEMPERATURE GRAPH OF DIFFERENT FORMS OF AVIAN TYPHOID

may run for an undetermined time, the bird either succumbing or apparently recovering as an individual to enter into the carrier stage. Such carriers are probably factors in the eventual recurrence of the disease after the outbreak has seemingly worn itself out.

A composite temperature graph of the types of fowl typhoid cases is presented in Figure 18.

The mortality attending avian typhoid varies according to conditions which are never constant. As an index to the mortality, Table IV lists the outbreaks with which this department came in contact during the studies on this subject. This table involves a total of 4,983 birds, the mortality before vaccination being 1,292 birds or 26 per cent; after vaccination, 137 birds or 3.8 flock per cent; the total representing a mortality of 1,432 birds or 29 flock per cent.

Table IV

Mortality in Fowl Typhoid

Flock Number	Loss Before Vaccination			Loss After Vaccination			Total Loss		
	Flock Total	Loss	Per Cent Loss	Flock Total	Loss	Per Cent Loss	Flock Total	Loss	Per Cent Loss
1	34	4	12	30	3	10	34	7	21
2	35	5	14	30	0	0	35	5	14
3	126	42	33	84	1	1	126	43	34
4	32	8	25	24	0	0	32	8	25
5	40	5	12	35	0	0	40	5	12
6	19	7	37	12	0	0	19	7	37
7	26	3	12	23	0	0	26	3	12
8	15	4	27	11	0	0	15	4	27
9	111	11	10	100	0	0	111	11	10
10	64	4	6	60	0	0	64	4	6
11	93	35	38	58	2	3	93	37	40
12	23	1	4	22	6	27	23	7	30
13	27	7	26	20	2	10	27	9	33
14	42	7	17	35	0	0	42	7	17
15	112	12	11	100	0	0	112	12	11
16	29	4	14	25	1	4	29	5	17
17	100	22	22	78	7	9	100	29	29
18	73	25	34	48	0	0	73	25	34
19	137	69	50	68	0	0	137	69	50
20	69	20	29	49	2	4	69	22	32
21	94	25	27	64	5	8	94	30	32
22	20	5	25	15	0	0	20	5	25
23	470	12	3	458	2	4	470	14	3
24	177	77	44	100	0	0	177	77	44
25	64	40	63	24	1	4	64	41	64
26	80	61	76	19	3	16	80	64	80
27	69	20	29	49	4	8	69	24	35
28	165	82	50	83	3	4	165	85	52
29	135	35	26	100	1	1	135	36	27
30	123	75	61	48	4	8	123	79	64
31	95	40	42	55	2	4	95	42	45
32	75	20	27	55	3	5	75	23	31
33	39	1	3	38	0	0	39	1	3
34	66	1	2	65	0	0	66	1	2
35	53	1	2	52	0	0	53	1	2
36	110	40	36	60	0	0	110	40	36
37	53	20	38	33	0	0	53	20	38
38	900	8	1	892	32	4	900	40	4

Table IV—Continued

Flock Number	Loss Before Vaccination			Loss After Vaccination			Total Loss		
	Flock Total	Loss	Per Cent Loss	Flock Total	Loss	Per Cent Loss	Flock Total	Loss	Per Cent Loss
39	89	15	18	75	11	15	89	26	29
40	49	35	72	15	1	7	49	36	73
41	70	35	50	--------	--------	--------	70	35	50
42	87	75	87	--------	--------	--------	87	75	87
43	65	30	46	35	6	17	65	36	55
44	31	26	84	5	1	10	31	27	87
45	42	7	17	35	1	3	42	8	19
46	30	13	43	17	1	6	30	14	47
47	72	12	17	60	1	1	72	13	20
48	50	35	70	--------	--------	--------	50	35	70
49	18	15	83	--------	--------	--------	18	15	83
50	25	25	100	--------	--------	--------	25	25	100
51	117	31	26	86	21	24	117	52	44
52	49	15	31	34	5	15	49	20	41
53	53	8	15	45	3	7	53	11	21
54	47	9	19	38	0	0	47	9	19
55	61	40	66	21	2	10	61	42	64
56	33	8	24	25	0	0	33	8	24

In estimating the flock mortality from this disease in France, d'Herelle (4) places the percentage from 40 to 70.

The spread of infection through the flock after the initial inception is probably very rapid. This spread is accentuated by the fact that the birds dwell in close contact; eat and drink from common containers; range on the same ground, and are prone to pick the carcasses of birds dying from the disease. The more important factors of dissemination of this and other avian diseases in the flock are:

Soil.—The dejecta from birds suffering from avian typhoid contains large numbers of virulent organisms expelled from the intestines with the fecal matter. It is the opinion of most investigators that the picking up of the organism from infected soil is the most common manner of the dissemination of typhoid within the flock.

After depositing the germ in the soil through the medium of the feces, the question arises as to the length of time which the organism will live and retain its vitality. It is evident that this period of time is not constant, and is governed somewhat by local conditions, and the virulence of the organism.

d'Herelle (4) cites the following instance of an outbreak of avian typhoid from infected ground:

> "In several tests, it has been shown that an isolated infected chicken yard, cleaned and left unoccupied for 6 to 8 months, still contains virulent germs, for when repopulated with chickens from a region free of the disease, the infection breaks out again within a few days among the new occupants."

In the course of investigation of field outbreaks, this department secured the following interesting history, which would substantiate the statement that E. sanguinaria might retain its virulence in the soil for the period of a year:

Avian typhoid had occurred at periodic intervals at this specific farm for five years, the owner selling the place and buying another. The new owner of the farm placed no birds on this farm for a year's time, but bought a flock from a farm in which the disease had never occurred, and placed them on the ground which had been used for range ground in the past. Avian typhoid appeared in this flock within a few weeks. This farm afterwards was probably the foci of an epizootic, which was eventually checked by vaccination.

The soil undoubtedly has exceptional advantages for harboring the germ, especially under barns and houses, where there is no possibility of the germicidal action of the sun's rays, and which places are favored by the birds for dust baths and for lying around.

Through the Egg.—The transmission of the disease through the egg to the young chick seems a possibility, although not a potent source of danger. This fact seems to be the opinion of the European school.

teHennepe (33) in a report on diseases at the World's Poultry Congress in Spain, dwells on infection through the egg as follows:

"Many chick diseases are spread by infected eggs. We often found Klein's disease in young chicks which were artificially hatched. This proves that the eggs were infected in the ovary of the mother hen."

At the same conference, Leynen (20) in a report from the laboratory of The Mnister of the Interior and Hygiene, Brussels, reports infection of eggs in an outbreak of paratyphoid in ducks and chicks, and asserts that the germ is present in the egg produced by adult carriers. Leynan further carried out examination of 200 new-laid eggs from fowls and ducks which had previously produced eggs from which chicks and ducklings hatched out and subsequently died of infection with paratyphoid B. These eggs had reached different stages of embryonic development, but Leynan was unable to isolate paratyphoid B. from any of the eggs or embryos, even when the latter arrived at full term.

It is a scientifically established fact that the germ of bacillary white diarrhea is transmitted from the adult bird to the young through the embryo. In the artificial infection work carried out by this department, it was possible to secure six eggs from birds artificially infected, and in the early stages of the disease. No organism which could be identified as E. sanguinaria was isolated from these eggs. Typhoid was later definitely confirmed in the birds laying these eggs. The possibility of the germ being transmitted to the young from the adult seems to be borne out by the fact that the culturing of 32 egg yolks or ovaries of hens dying from the disease showed 20 positive for E. sanguinaria; the testicles of 8 male birds cultured showed 5 positive. The chances of birds suffering from infection with avian typhoid delivering a large number of eggs seems remote, for the toxemia is so acute that body metabolism is upset, and the bird goes off lay soon after contracting the disease. As previously mentioned, the disease was not found prevalent among chicks in the field where infection was present in adults.

Drinking Water.—The drinking water is a common source of the spread of avian typhoid in an infected flock. The infection of the water is generally accomplished by the depositing of infected fecal material in the drinking water. In artificial infection work the disease was readily produced by depositing such fecal material in the drinking water of well birds.

Nasal Secretions.—The extent that nasal secretions or excretions convey this disease to the drinking water is unknown, but it is probable that such method of transmission is remote, as it is hardly possible that the upper air passages are seats of infection. Attempts in the laboratory to produce the disease by allowing well birds to drink the water used by typhoid cases failed.

ARTIFICIAL INFECTION

Artificial infection work in the laboratory with the organism of avian typhoid represents confirmatory studies of the observations made in the field during these investigations. Its objective point was a more detailed study of the specific disease than opportunities in the field afforded; the tracing of the various possible avenues of infection; observations of the development of symptoms; studies of the changing condition of the blood from inception of the disease until death; noting the sereological conditions in the body of the host; the study of the morbid anatomy of birds succumbing to the disease, and the development of such control measures as to keep this devastating contagium from making poultry production in North Carolina unprofitable.

Birds used for infection purposes were healthy, sound adults or chicks, care being taken to make the studies representative of the order Gallinæ. All birds were tested prior to inoculation as to the normality of body temperature, and for possible existing bacteremic conditions. Pre-inoculation blood studies were made on two successive days when hematological studies were made. The birds were closely watched for physical depression, the condition of the discharge, and other signs which might be indicative of body change.

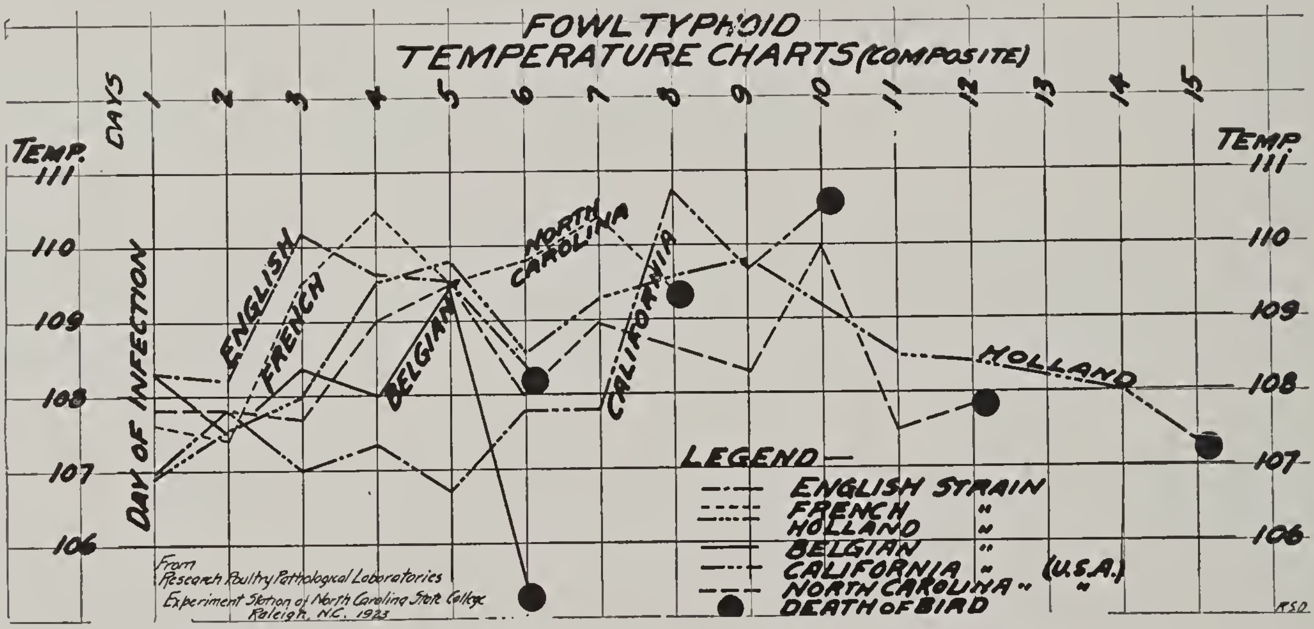

FIG. 19.—COMPOSITE TEMPERATURE CHART OF FOWL TYPHOID IN BIRDS INFECTED BY E. SANGUINARIUM FROM DIFFERENT COUNTRIES

Little difficulty was experienced in infecting birds with E. sanguinaria (Figure 19). The department was fortunate in being able to secure original isolations of the causative organism at frequent intervals from birds dying in the field, and the strains used in artificial infection work were, for the greater part, of such character. Observations in this work tended to indicate that the virulence of E. sanguinaria was considerably lessened by transferring for several generations on artificial media. This observation is borne out by other investigators.

Moore (23) in his original studies on the disease successfully produced fowl typhoid by infecting the birds intravenously; by feeding cultures of the organism, and by feeding the viscera and cultures. Moore obtained a high death rate in his experimental inoculations. Taylor (30) produced the disease in well fowls by the intravenous inoculation method; by feeding cultures, and by feeding portions of the viscera of birds dying of the disease.

The efforts of this department in artificial infection were to infect through such natural channels as would be possible in the field. The intravenous method, and that of feeding the viscera, were not attempted, the work of the above-mentioned writers indicating that the disease was readily produced through such means of infection.

By infecting the drinking water with bacterial cultures of E. sanguinaria, typical cases of avian typhoid were regularly produced. Studies of infections produced through this manner of infection does not indicate that there is any tendency to extend or shorten the period of disease as noted in the field. A typical case of avian typhoid as produced through this manner of infection is listed below:

Subject.—Barred Plymouth Rock pullet.

Source.—Poultry hospital.

Condition on Infection.—Normal bird in excellent vigor.

Method of Infection.—3 cc. of a 24 hour, bacillary saline emulsion of American sanguinarium 63 placed in drinking water at 11 a. m., February 28, 1923.

CLINICAL DATA

Date	*Respiration*	*Temperature*	*Remarks*
Feb. 26	30	107.8	Pre-inoculation
27	30	107.8	Pre-inoculation
28	30	107.0	Day of inoculation
Mar. 1	32	107.2	Bird normal
2	30	106.6	Dejecta watery
3	34	107.8	Dejecta watery
4	30	107.8	Bird dejected
5	34	110.8	Sulphurous discharge
6	30	108.9	Bird depressed
7	29	109.6	Bird depressed

The bird died 12:30 p. m. March 7. E. sanguinaria recovered from heart, liver, spleen, kidneys, lungs, and bone marrow.

The concensus of opinion of various investigators as to the transmission of this disease is that the discharge from the bowel contains large numbers of virulent germs, which through many ways might find their way into the alimentary tract of well birds, thereby producing the disease. The contamination of drinking water by active cases of avian typhoid, or by carriers, or the soiling of food material by the same, are probably the means of many infections.

By adding 5 grams of fecal material from a typical case of avian typhoid to the drinking water of a well bird, a typical and fatal case of avian typhoid was produced, with the following hospital chart:

Subject.—White Leghorn hen.

Source.—Central plant.

Condition on Infection.—Bird normal.

Method of Infection.—About 5 grams of fecal material from a bird showing typical symptoms of avian typhoid was placed in the drinking water of this bird on March 1, 1923, at 10 a. m. At the time of dejecting, the typhoid case showed a temperature of 110 degrees.

CLINICAL DATA

Date	*Respiration*	*Temperature*	*Remarks*
Feb. 27	35	107.8	Pre-inoculation
28	33	107.0	Pre-inoculation
Mar. 1	33	107.5	Day of inoculation
2	32	108.2	Bird normal
3	30	110.2	Whitish dejecta; depression
4	28	109.8	Watery discharge
5	28	109.7	Sulphurus discharge
6	30	108.0	Extreme depression
7	30	109.0	Extreme depression
8	29	108.6	Extreme depression
9	30	108.2	Extreme depression
10	28	110.0	Partial condition of coma
11	27	107.5	Partial condition of coma
12	20	107.0	Partial condition of coma

The bird died during the morning of the 12th. E. sanguinaria recovered from the liver, spleen, kidneys and bone marrow. Heart and lung cultures negative.

The disease was successfully produced by subcutaneous inoculation as shown in the following clinical study:

Subject.—Rhode Island Red hen.

Source.—Central plant.

Condition on Infection.—Bird normal.

Method of Infection.—2 cc. of a 24-hour bacillary saline emulsion of American sanguinarium 67 given subcutaneously March 5, 1923, at 10:30 a. m.

CLINICAL DATA

Date	*Respiration*	*Temperature*	*Remarks*
Mar. 3	38	106.4	Pre-inoculation
4	36	107.0	Pre-inoculation
5	42	108.0	Day of inoculation
6	35	108.0	Bird normal
7	42	108.8	Slightly depressed
8	36	108.0	Slightly depressed
9	36	108.8	Slightly depressed
10	42	108.3	Bird dejected
11	36	109.5	Watery discharge
12	31	109.3	Watery discharge
13	30	108.8	Watery discharge
14	34	108.1	Watery discharge
15	30	107.8	Sulphur colored discharge
16	36	108.6	Bird depressed
17	30	107.8	Bird depressed
18	30	107.8	Bird depressed
19	30	107.8	Bird depressed
20	30	108.2	Bird depressed

The bird died at noon on the 20th. E. sanguinaria isolated from the heart, liver and spleen.

The most successful method of infecting birds with avian typhoid in these experiments was by infection per orum. In this method of infection the period of incubation seemed relatively constant, and this method was used in blood study work in order to secure a maximum chance of take, and eliminate the necessity of discarding pre-inoculation data on account of failure of the bird to contract the disease. A clinical chart, which is typical of a bird infected through such an avenue is given:

Subject.—Rhode Island Red cock.

Source.—Central plant.

Condition on Infection.—Bird normal.

Method of Infection.—3 cc. of a 24-hour bacillary saline emulsion of Wake County strain of avian typhoid fed per orum, March 20, at 10:00 a. m.

CLINICAL DATA

	Temperature		
Date	*A.M.*	*P.M.*	*Remarks*
Mar. 19	106.9	106.6	Pre-inoculation
20	107.6	106.6	Day of inoculation
21	107.0	106.0	Bird normal
22	107.2	106.6	Bird normal
23	107.8	108.6	Bird depressed
24	109.4	108.5	Bird depressed
25	109.6	109.6	Sulphur colored discharge
26	109.6	109.8	Sulphur colored discharge
27	109.4		

The temperature was taken at 8:00 a. m. on the 27th; the bird died at 8:30. E. sanguinaria recovered from heart, liver, spleen, kidneys, lungs, and bone marrow.

A table listing the results of artificial infection is set forth in Table V.

Table V

Clinical Studies of Artificially Infected Birds

Breed	Sex	Strain Used	Infected per—	First Symptom, Days	Duration to Death, Days	Duration to Recovery, Days	No Symptoms, Days	Temperature		Respiration		Remarks
								Max.	Min.	Max.	Min.	
W. Leghorn	F	American	Drinking water	6	8			112.0	105.6			
R. I. Red	F	American	Fecal matter				9	107.8	105.6	50	41	
R. I. Red	F	Holland	Drinking water	3		10		108.0	106.0	48	41	Positive bacteremia on 3d day
Ancona	M	English	Drinking water	12	30			110.6	107.0	38	20	Organism recovered from blood of Bird 3, which threw off the disease
R. I. Red	F	American	Drinking water	4	12			109.9	107.2	45	34	
W. Leghorn	F	American	Drinking water	6	25			110.5	106.6	45	36	
W. Leghorn	F	American	Fecal matter	3	12			110.2	107.0	33	20	
B. P. Rock	F	American	Drinking water	6	8			110.8	106.6	34	29	
R. I. Red	F	American	Sub. inoculation	3	19			109.5	106.4	42	30	
W. Leghorn	M	American	Fecal matter	5		17		108.5	107.4	48	30	
W. Leghorn	F	English	Drinking water	4		16		108.4	107.0	42	30	
Mongrel	F	French	Orum	3	7			110.5	107.4	28	20	
B. P. Rock	F	American	Drinking water	6		9		108.5	107.0	38	28	
B. P. Rock	F	English	Drinking water	4		12		109.5	106.2	40	30	
W. Leghorn	F	American	Orum		8							
W. Leghorn	F	English	Orum	4	6			109.5	106.9	38	30	Bacteriophage two plus virulent for E. sanguinaria; isolated on 6th day
W. Leghorn	F	Belgian	Orum				8	107.6	106.3	42	36	
W. Leghorn	M	American	Orum				8	107.6	106.5	42	40	
R. I. Red	F	American	Orum				8	107.0	106.0	45	33	
Ancona	F	American	Orum	6		11		109.5	105.8			
R. I. Red	F	Belgian	Orum	7		11		108.5	105.8	48	27	
W. Leghorn	F	Belgian	Orum	3		12		109.2	106.7	45	30	
W. Leghorn	F	Belgian	Orum	5		11		109.5	106.7	54	30	
W. Leghorn	F	Belgian	Orum	3	6			109.5	102.2	63	30	
R. I. Red	F	Belgian	Orum	3		8		108.3	105.8	48	30	
W. Leghorn	F	American	Orum				11	107.6	106.2	40	33	
R. I. Red	F	American	Orum				9	107.0	106.2	44	34	
Mongrel	F	American	Orum				9	107.2	106.6	25	17	
W. Leghorn	F	American	Orum	3		15		109.0	107.2	55	36	
W. Leghorn	F	American	Orum				8	107.0	106.2	40	36	
W. Leghorn	M	American	Orum				8	108.2	107.2	52	32	
W. Leghorn	F	French	Orum	5	9			108.0	106.5	38	23	
B. Orpington	F	French	Orum	4	6			110.0	105.0	41	34	
W. Leghorn	F	French	Orum				12	107.9	106.0	41	34	
R. I. Red	F	French	Orum				11	107.3	104.0	44	36	
W. Leghorn	F	English	Orum				11	107.8	106.4	27	18	
R. I. Red	F	English	Fecal matter				11	107.8	106.8	40	27	

W. P. Rock	F	American	Orum	6	9	----	----	109.8	106.2	----	----	
W. Wyandotte	F	American	Orum	----	----	----	7	107.4	106.2	----	----	
R. I. Red	F	American	Orum	4	8	----	----	109.8	106.6	----	----	
W. Leghorn	F	American	Orum	----	----	----	6	108.3	105.3	----	----	
W. P. Duck	F	American	Orum	5	----	9	----	108.7	106.2	----	----	
Guinea	M	American	Orum	2	5	----	----	109.5	107.0	----	----	
R. I. Red	F	American	Orum	6	9	----	----	110.5	106.6	----	----	
R. I. Red	F	American	Orum	6	12	----	----	109.7	104.5	----	----	Bird received two vaccinations three years ago
B. P. Rock	F	American	Orum	----	----	----	7	107.2	106.0	24	20	Bird received two vaccinations three years ago
W. Leghorn	F	American	Orum	4	5	----	----	113.0	106.6	----	----	
Mongrel	M	American	Orum	4	----	23	----	109.9	106.5	----	----	
Mongrel	F	American	Orum	----	----	----	10	107.8	106.4	----	----	
W. Leghorn	M	American	Orum	5	8	----	----	112.2	106.9	----	----	
Mongrel	F	American	Orum	7	----	11	----	109.1	106.2	----	----	
Pigeon	F	American	Orum	3	----	15	----	111.5	107.0	----	----	
Pigeon	F	American	Orum	4	----	15	----	110.5	105.9	----	----	

CHICKS

W. Leghorn	----	American	Drinking water	----	----	----	7	106.7	105.0	----	----	Three weeks old
W. Leghorn	----	American	Drinking water	3	6	----	----	109.7	106.3	----	----	Three weeks old
W. Leghorn	----	American	Orum	5	6	----	----	107.6	106.0	----	----	Three weeks old
W. Leghorn	----	American	Orum	3	6	----	----	107.6	104.0	----	----	Three weeks old
W. Leghorn	----	American	Subcutaneous	----	----	----	7	107.5	104.2	----	----	Three weeks old
W. Leghorn	----	American	Subcutaneous	----	----	----	7	106.4	104.0	----	----	Three weeks old
W. Leghorn	----	American	Drinking water	----	----	----	7	106.3	105.0	----	----	Four weeks old
W. Leghorn	----	American	Drinking water	3	6	----	----	109.7	106.3	----	----	Four weeks old
W. Leghorn	----	American	Orum	----	----	----	7	107.0	106.0	----	----	Four weeks old
W. Leghorn	----	American	Orum	----	----	----	7	107.6	104.0	----	----	Four weeks old
W. Leghorn	----	American	Subcutaneous	----	----	----	7	107.5	104.2	----	----	Four weeks old
W. Leghorn	----	American	Subcutaneous	----	----	----	7	105.6	104.0	----	----	Four weeks old

Six chicks, one week of age, infected: two per drinking water; two per orum, and two by subcutaneous inoculation. Five died from seven to nine days; one of birds infected through drinking water did not contract the disease.

Six chicks, two weeks of age, infected as above: these chicks did not contract the disease.

Strictly speaking, avian typhoid should be classified as an infectious disease, which, according to Kolmer (19), is a disease which is the result of indirect transmission.

Such a type of disease, according to the same authority, passes through the following stages:

1. The period of incubation, which begins at the time of infection, and ends with the development of the earliest symptoms, during which time the invading parasites are multiplying in the tissues of the host. During this stage no symptoms, or only those of a purely local nature, are present. The period varies considerable in different infections, and to a lesser degree in different individuals having the same infection.

In artificial infection experiments with avian typhoid, 35 out of 39 adult birds contracting the disease showed clinical symptoms in from 3 to 6 days; three of the remaining ones did not develop symptoms until the 7th day, and the fourth one did not show symptoms of the disease until the twenty-first day. The last bird possessed high natural resistance to avian typhoid, as 30 days elapsed before the bird finally succumbed.

2. The period of prodromal symptoms, characterized by systematic disturbances of a relatively mild type, due to the diffusion of bacteria and their products into the general circulation, and their wide-spread effect upon the body cells in general.

Sufficient data was not accumulated in these artificial infections to arrirve at a definite conclusion as to the stage of the disease when a bacteremia begins and ends. Successful isolations of E. sanguinaria from the blood current were made in two instances, one 3 days after infection, and the other on the 6th day. All birds infected were tested daily for a bacteremia, but negative results were obtained except in the above mentioned cases. There is probably an overlapping in these two periods in infection with avian typhoid, varying undoubtedly, with the severity of infection and the resistance of the bird. Through the latter part of the period of incubation, until the period of fastigium or high fever, there is a material derangement of the bowel, the dejecta being watery in consistency, and sulphurus in color, the coloration being due to bilirubin and biliverdin.

3. The period of Fastigium or high fever, in which the disease is at its height.

In avian typhoid, this period finds its inception and duration in the resistance of the individual bird, and the severity of infection. In birds infected artificially, the third period could be designated as commencing from the third to the sixth day, the birds succembing, as a rule, in less than ten days. As indicated by Kolmer, the period of Fastigium, during which the birds which succumb to the disease usually die, is one in which the host is overwhelmed with the toxic split portion of the newly formed bacterial protein. The fever is the resultant of disturbance in the circulatory, respiratory, and heat regulating mechanism, and in general metabolism.

4. The period of decline, during which the patient is gradually overcoming the infection, and amelioration of the symptoms takes place.

5. The period of Convalescence, in which the host gradually overcomes the effects of the disease and returns to health.

In periods 4 and 5 in avian typhoid, the transformation from period 3 is relatively abrupt, birds seemingly overcoming the effects of the disease over night, a return to normal condition ensuing.

Salmon in his original experiments on avian cholera, arrived at the conclusion that 18 out of 100 birds possessed natural immunity to the disease.

On the same basis of reasoning, eliminating attempts to infect immunized birds, these experiment would show that 30 out of 100 birds are naturally immune to avian typhoid, or a figure fairly close to Salmon's estimate.

These figures compare favorably to the mortality index of outbreaks in the field as shown in Table IV, in which the flock mortality before vaccination was 26 per cent; after vaccination 3.8 per cent; with a total mortality of 29 per cent, which would give the per cent of birds naturally immune up to the time of vaccination of 74 per cent.

teHennepe (33) is inclined to consider the disease as very acute, and sets the duration from one-half day up to five days, with chronic cases occurring rarely, such cases being important from a pathological and anatomical standpoint. teHennepe's conception would tend to concentrate the stages of the disease as outlined above.

Truche and Staub (32) held that the duration of the disease is variable, but generally runs its course in from 2 to 3 days, which would have the same effect in concentrating the periods as teHennepe's conception.

HEAT ELIMINATION IN FEBRILE DISEASES

The different tissues of the bird's body eliminate heat in amounts corresponding almost precisely with the intensity of their metabolism in health, but this equilibrium may be disturbed in disease. The body temperature deviation due to age, sex, exercise, season, climate or feathers rarely under normal conditions amounts to more than one or two degrees, and is temporary in nature. Increased body temperatures may also be caused by a rise of outside temperature (1). This change is usually associated with increased frequency of heart action and with respiration, fatigue and possible loss of consciousness, and death may result in a short time. The diurnal variation of the normal temperatuere of a fowl ranges from 104.5 degrees F. between midnight and 3 a. m., to 107.3 degrees F. between 12 mid-day and 5 p. m. The temperature of the larger birds as turkeys and geese are slightly lower than that of hens, while the temperature of the smaller birds, as pigeons, is slightly higher (14).

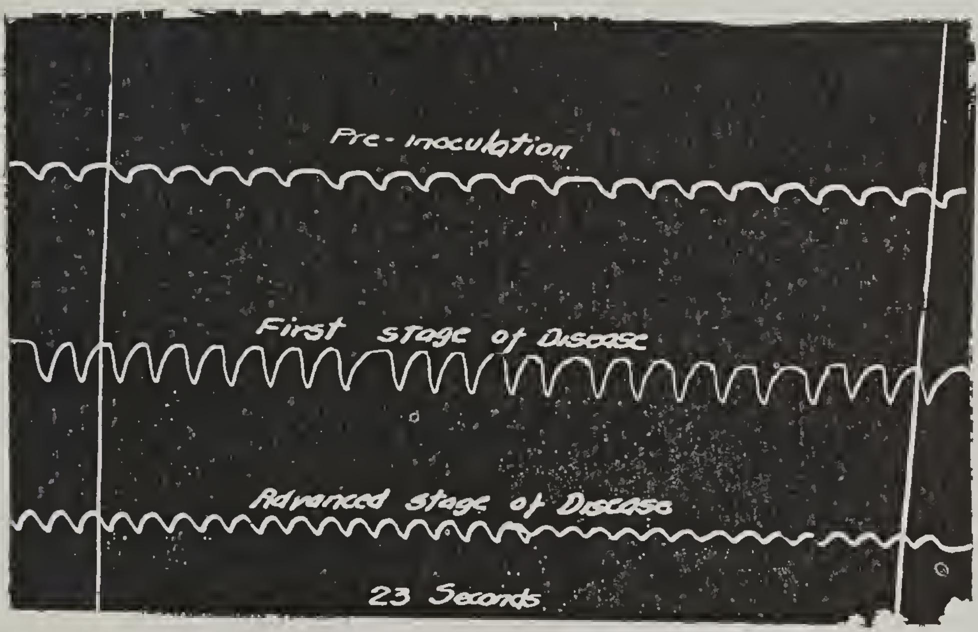

FIG. 20.—KYMOGRAPHIC TRACING OF NORMAL RESPIRATION OF BIRD, AND SAME BIRD IN EARLY AND IN ADVANCED STAGES OF FOWL TYPHOID

Hyperthermy, or the condition known as fever, is represented by a complex of symptoms of which a decided and lasting elevation of body temperature is characteristic. This condition is not due to food, exercise, or environmental temperature, but is usually caused by the breaking down of the functioning of the heat regulating mechanism. This condition follows the entrance into the body of pathogenic bacteria and their toxic substances. Accompanying this condition is thirst, weakness, and alteration in body secretions. The fever may begin abruptly with a chill, or it may be intermittent. Hirsch, Muller, and Rolly (1) have advanced the view that fever results in consequence of a derangement of metabolic condition of tissue cells by poisonous substances. This theory is well adapted to those febrile conditions following the entrance of pathogenic bacteria into the system, and also admits of the possibility that there are heat regulating centers in the brain which may be upset by the toxemia produced by infection. In the temperature studies in this investigation the temperatures of infected birds ranged from normal to 113 degrees F.

Heat is dissipated from the body of the bird, to a limited extent, by the skin through the process of radiation and convection, though the feather coat retards this process, and to a greater extent by the way of the pulmonary passage, the heat being transferred to the inspired air. The respired air is made to oscillate back and forth across the moistened mucous surface, and some bound heat is also eliminated by the droppings. Hence, respiration is increased when excess of heat is present in the body. The normal physiological respiratory centers in the brain govern both the length and depth of breathing (1), thus determining lung ventilation. In Figure 20 there is shown a kymographic tracing of the respiratory movements in an artificially infected case of avian typhoid. It is noted that in the preinoculation study, the hen breathed 19 times in 23 seconds and that in the initial stages of the disease the response is a greater depth in the respiratory movement (20 in 23 seconds) which constitutes labored breathing. In the later stages the breathing is again more shallow and accelerated, amounting to 27 respiratory movements in 23 seconds. In many instances there is a serous effusion into the lung alveoli which further complicates the process of heat dissipation. The pre-inoculation respiration in the figure shown is above normal, but the three readings are comparable as the tracing was taken under the same conditions, except for disease advancement. Great care is always exercised to avoid exciting the fowl, as under the strain of excitement and fear the respirations are sure to be increased.

The increase in respiration, as the result of disease, is due to a stimulation of the heat dissipating center. The normal respirations, as determined for utility cocks, as Rhode Island Reds and Plymouth Rocks, is 20 times per minute, and for hens of similar breeds, 36 times a minute (14). In the artificial infections studied the respiration was irregular in frequency, being recorded in one instance as high as 63 per minute.

PATHOLOGICAL ANATOMY

The anatomy of birds dying from avian typhoid suffers considerable pathological alteration.

Moore (23) in his original work on this disease states that the only constant lesions in fowls which contracted the disease naturally, as well as those fed upon its virus, were in the liver and blood. He makes note of the fact that in his investigations the affections of the intestinal tract were rare; the kidneys pale but streaked with reddish lines, due to injection of blood vessels; spleen rarely discolored or engorged with blood; lungs pale but normal; heart pale with grayish points; lymphatic glands not appreciably enlarged, but there were alterations in the blood composition which were constant and typical of the disease.

Taylor (30) reports the specific morbid conditions as consisting of an enlarged liver containing necratic areas, an enlarged spleen, and a general anemic condition of the serous and mucus membranes, together with a marked increase in leucocytes and a corresponding relative decrease in red cells.

Hadley (10) designated the pathological changes as highly variable; exudative or hemorrhagic symptoms or both are common; pericardial exudate common; leukemic condition often present; degeneration of the liver capsule common, with the liver enlarged, and the skin of the breast reddened along the keel.

The data on pathological anatomy incorporated in this article was secured from birds dying in the field where the disease was contracted under natural conditions, and from the poultry hospital where birds were artificially infected. The pathological anatomy is divided into gross anatomy and microscopic anatomy.

GROSS ANATOMY

The face, comb, and wattles of birds dying of avian typhoid is usually pale, due to a weak circulation and the leukemic conditions of the blood. No other external sign is noted except the possible soiling of the vent fluff with a sulphur colored fecal discharge.

Heart.—The blood vessels of the heart are engorged with blood; hydropericardium is frequently present, this being noted in 11 out of 129 birds posted or in 8 per cent. This condition is probably caused by an injury to the endothelial cells, due to acute toxemia produced by the disease. Relative weights of the heart to the body weight is shown in table and compared to the normal as established by Latimer (21) in Figure 21. The number of instances in which sanguinaria was recovered from the heart blood is shown in Ttable VI.

Table VI

Recovery of E. Sanguinaria From Birds Dying From Fowl Typhoid

Source of Culture	*Number of Birds Cultured*	*Number of Cultures Positive*	*Number of Cultures Negative*	*Percent of Cultures Positive*
Heart	129	112	17	87
Liver	129	125	4	97
Spleen	110	102	8	93
Kidneys	84	72	12	86
Lungs	52	42	10	81
Bile	12	3	9	25
Tissue	19	2	17	11
Brain	24	10	14	42
Bone marrow	57	53	4	93
Reproductive organs, ovaries and egg yolks	32	20	12	63
Tests	8	5	3	63

Liver.—The liver is enlarged, streaked, and friable, showing gross indications of congestion. Only very rarely were necrotic areas observed. Out of 129 birds posted, 101, or 79 per cent, showed abnormalities of the liver. The distension of the individual cells and congestion of the veins and arteries account for the enlarged and friable condition of the organ. The relative weights of the liver to the weight of the body is shown in Table VII. Frequency of the recovery of E. sanguinaria from the liver is shown in Table VI. The relative weight of the liver in avian typhoid to that of the normal liver in a bird of the same size is shown in Figure 22.

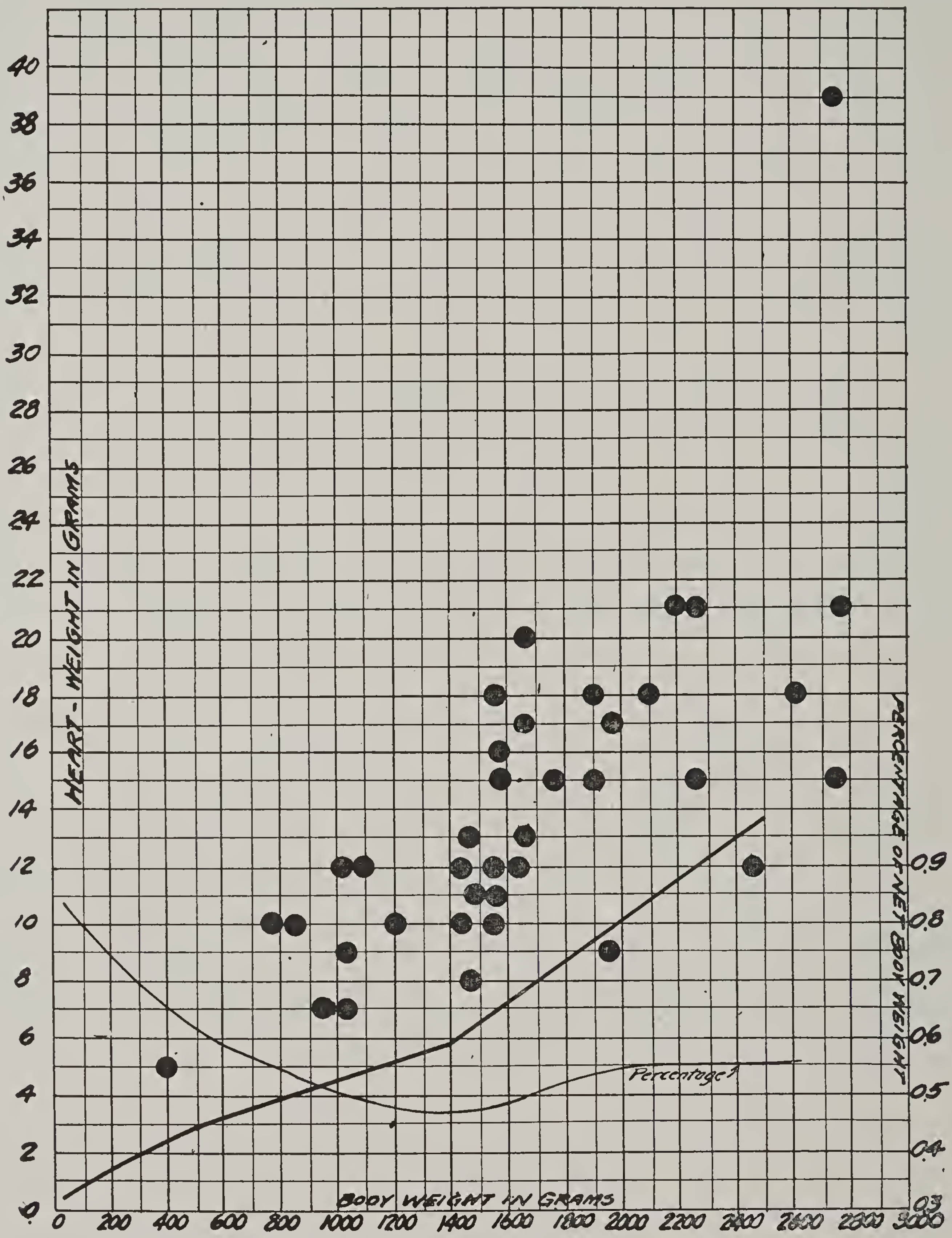

FIG. 21.—GROSS WEIGHT OF HEART IN NORMAL BIRD, AND IN TYPHOID BIRD OF SAME WEIGHT

Spleen.—The spleen presented abnormal conditions in gross in 89 out of 129 birds posted, or in 69 per cent of the total. The most frequent condition noted was enlargement, though in six instances the organ was mottled. One instance showed a sub-sized spleen. The enlargement of the spleen is due to engorgement with blood. The relative weight of the spleen in typhoid to body weight is shown in Table VII, and in comparison to the weight of the organ in a normal bird of the same weight in Figure 23. The frequency of recovery of E. Sanguinaria from the spleen of diseased birds is shown in Table VI.

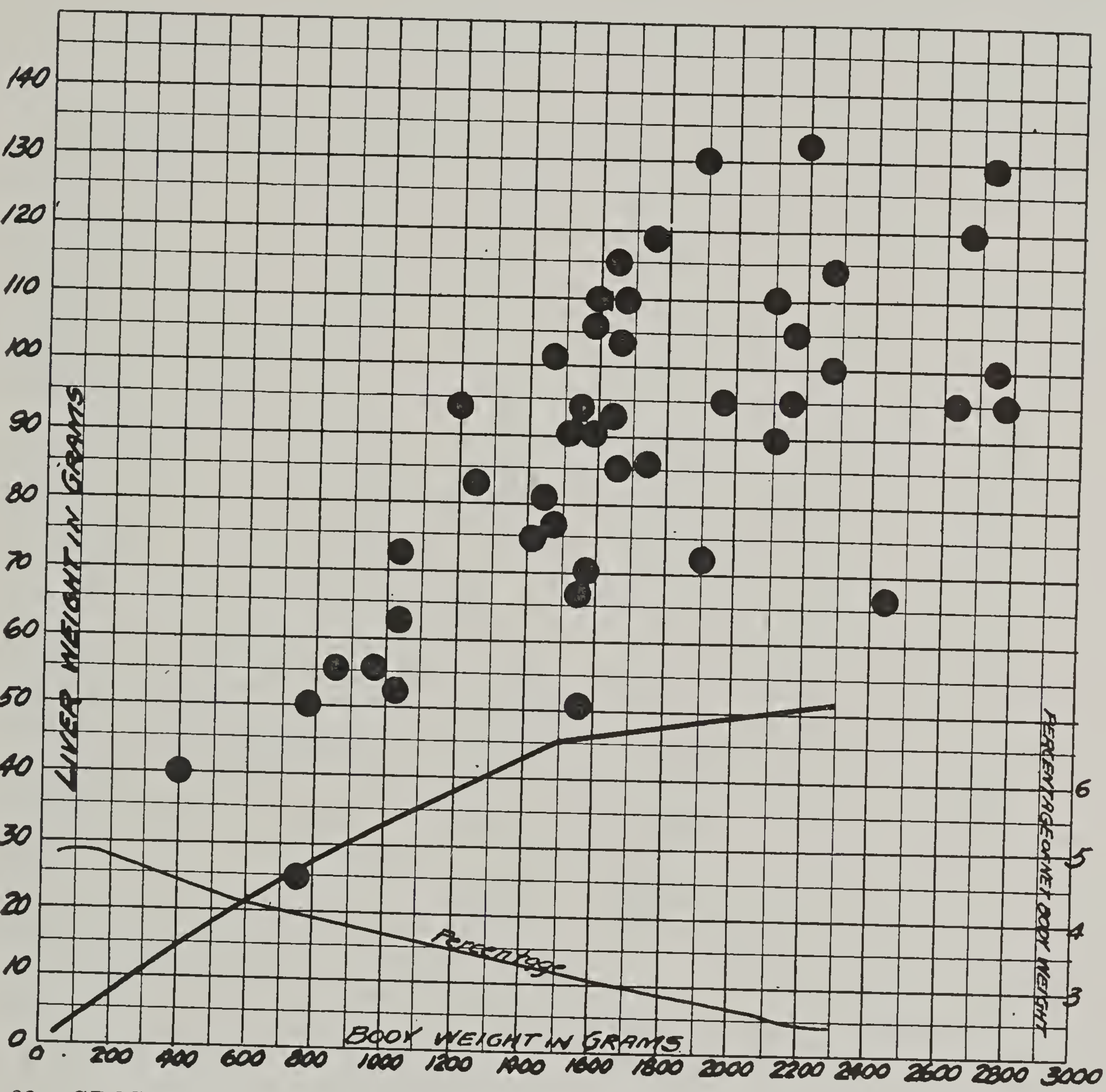

FIG. 22.—GROSS WEIGHT OF LIVER IN NORMAL BIRD, AND IN TYPHOID BIRD OF SAME WEIGHT

Kidneys.—The kidneys presented abnormal gross appearance in 85 out of 129 birds posted, or in 66 per cent of the total. The abnormalities most frequently noted would indicate congestion and cloudy swelling, although in some instances the kidneys were streaked and spotted. The enlargement and congestion of the kidneys is due to increased blood in the organ. Relative weights of the kidneys in typhoid to the body weight of the bird is shown in Table VII, and in comparison to the weight of the organ in a normal bird of the same weight in Figure 24. The frequency in which E. Sanguinaria was recovered from the kidneys in diseased birds is noted in Table VI.

Lungs.—The condition of the lungs in birds dying from avian typhoid varied, probably due to the severity of the attack of the disease. Twenty of 54 birds posted, or 37 per cent of the total, showed a serous condition of the lungs. The weight of the lungs, and body weight of the dead bird is shown in Table VII, and the frequency of recovery of E. Sanguinaria from the lungs of birds dying from avian typhoid is shown in Table VI.

Gall Bladder.—The gall bladder may or may not be distended. If distended the condition is due to the accumulation of bile stored after active digestion ceases as a result of the disease. Frequency of recovery of sanguinarium from the bile is noted in Table VI.

Intestines.—A condition of suggillation of the intestinal mucosa and bowel content may be present (Figure 25). This was found in birds posted in the ratio of 39 to 31. The mucosa is usually thickened. The hemorrhagic condition is due to injury of the capillary walls by the toxin given off by the organisms. Twenty-five shows such a condition of the intestines as found in avian typhoid.

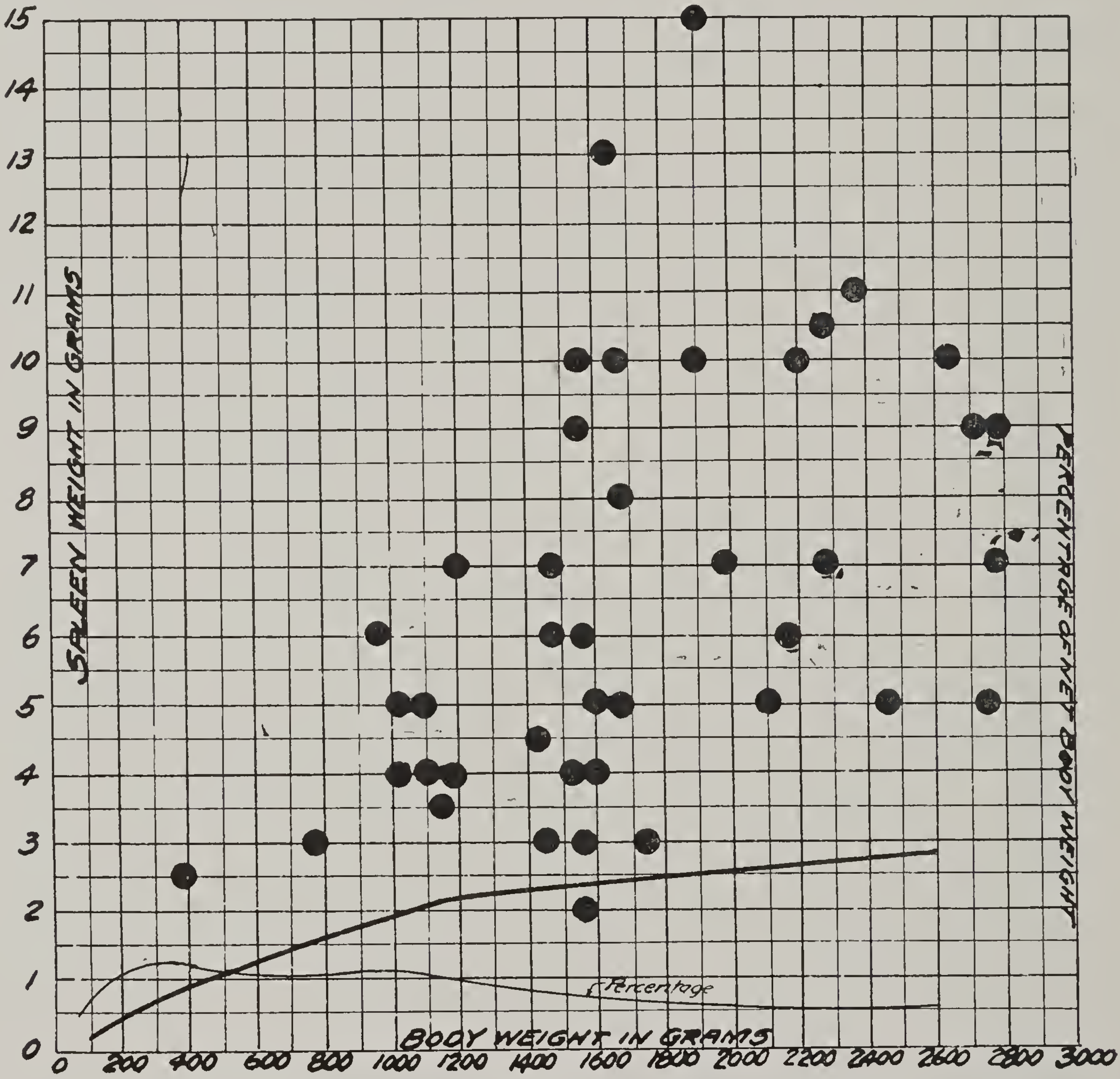

FIG. 23.—GROSS WEIGHT OF SPLEEN IN NORMAL BIRD, AND IN TYPHOID BIRD OF SAME WEIGHT

Reproductive Organs.—In the case of the laying hen, the disease is usually so sudden that the ovary still contains yolks well along in their development. There is no visible alteration of the testes. The frequency of recovery of E. sanguinaria is shown in Table VI.

The disease is usually accompanied by a loss of body weight. This emaciation is relative to the duration of the disease, and the severity of the attack. The physiological reason for this loss of weight is probably due to nutritional disturbance, or to metabolic upset, due to the toxic condition of the blood which is present in this disease.

Tabulations of the loss of body weight of birds artificially infected are listed in Table VIII.

Table VII

Body and Visceral Weights of Birds Succumbing to Artificial Infection With E. Sanguinaria

(Numbers Refers to Weight in Grams)

Breed	Body Weight	Heart		Liver		Spleen		Kidneys		Lungs	
		Weight	Per Cent Body Weight	Weight	Per Cent Body Weight	Weight	Per Cent Body Weight	Weight	Per Cent Body Weight	Weight	Per Cent Body Weight
B. P. Rock	1735	15	0.86	85	5.00	3	0.17	------	------	------	------
R. I. Red	1575	15	.95	50	3.17	2	.13	------	------	------	------
B. P. Rock	1420	12	.85	80	5.63	5	.35	------	------	------	------
B. Orp. (Pul.)	1020	12	1.17	72	7.06	5	.49	------	------	------	------
R. I. Red	1900	15	.79	130	6.84	15	.79	------	------	------	------
B. P. Rock	1520	10	.66	90	5.92	4	.26	------	------	------	------
B. P. Rock (Pul.)	770	------	------	25	3.25	------	------	------	------	------	------
R. I. Red (Ckl.)	1650	20	1.21	115	6.97	10	.61	------	------	------	------
B. P. Rock (Ckl.)	850	10	1.18	55	6.47	------	------	------	------	------	------
B. Orp.	985	7	.71	55	5.58	6	.61	------	------	------	------
B. P. Rock (Pul.)	787	10	1.27	50	6.25	3	.38	------	------	------	------
R. I. Red	2275	21	.92	114	5.01	7	.31	------	------	------	------
R. I. Red	2170	------	------	105	4.84	6	.27	------	------	------	------
R. I. Red	2770	------	------	120	4.33	7	.25	------	------	------	------
R. I. Red	2115	------	------	95	4.49	------	------	------	------	------	------
R. I. Red	1670	------	------	110	6.59	8	.48	------	------	------	------
B. P. Rock	1650	7	.42	85	5.15	5	.30	------	------	------	------
W. Leghorn	1460	8	.55	102	7.00	3.5	.24	------	------	------	------
Mongrel	1950	9	.46	95	4.87	4	.21	------	------	------	------
R. I. Red (Pul.)	400	5	1.25	40	10.00	3	.75	------	------	------	------
B. Orp. (Pul.)	1200	10	.83	94	7.83	7	.58	------	------	------	------
W. P. Rock	3947	32	.81	157	4.00	12	.31	15	.38	------	------
R. I. Red	1590	16	1.01	106	6.66	6	.38	20	1.26	11	.69
R. I. Red	2200	21	.95	132	6.00	10	.45	23	1.05	20	.91
R. I. Red	1460	13	.89	77	5.27	7	.48	25	1.71	10	.69
R. I. Red	1580	------	------	70	4.43	3	.19	------	------	18	1.14
W. Leghorn	1655	17	1.03	104	6.28	29	1.75	21	1.27	21	1.27
R. I. Red	1900	18	.95	72	3.79	10	.53	28	1.47	15	.79
R. I. Red	2605	18	.69	95	3.65	10	.38	22	.84	22	.84
B. P. Rock	2100	18	.86	90	4.29	5	.24	28	1.33	16	.76
Guinea	1080	12	1.11	52	4.81	5	.46	15	1.39	15	1.39
R. I. Red	2725	15	.55	100	3.67	5	.18	20	.73	16	.59
R. I. Red	1475	11	.76	71	4.81	6	.41	17	1.15	13	.88
R. I. Red	2780	21	.76	95	3.42	9	.32	------	------	------	------
R. I. Red	1985	17	.86	119	5.99	7	.35	20	1.01	15	.76
R. I. Red (Cock)	2724	39	1.44	129	4.71	9	.33	23	.84	28	1.03
W. Leghorn	1530	12	.78	67	4.38	4	.26	14	.91	10	.65
R. I. Red	3195	13	.75	105	6.08	5	.28	------	------	13	.77
R. I. Red	2285	15	.87	100	5.70	11	.63	------	------	------	------
Ancona	1117	8	.71	84	7.52	4	.35	17	1.52	11	.99
R. I. Red	1542	11	.71	94	6.10	9	.58	23	1.49	15	.98
Ancona	1005	9	.90	65	6.47	4	.40	17	1.69	9	.90
B. P. Rock	1610	12	.75	95	5.90	5	.31	12	.75	14	.87
Mongrel	1630	13	.80	110	6.75	13	.80	24	1.47	16	.97
Mongrel (Cock)	1558	18	1.16	91	5.84	10	.64	22	1.41	20	1.28
B. P. Rock	2460	12	.49	67	2.72	5	.20	------	------	12	.49
W. Rock	1415	10	.70	75	5.30	3	.21	------	------	15	1.06
Mongrel	2100	18	.86	110	5.24	5	.24	------	------	------	------

MICROSCOPIC ANATOMY

Microscopic anatomy as found in study of visceral organs of birds dying from avian typhoid under artificial infection conditions agreed, in general, with the microscopic anatomy as described in the typical field study as presented under that heading.

Heart.—The lesions of the heart and circulatory structures are considered as of a secondary nature, and may be a condition of cloudy swelling, congestion, or even inflammation giving a parboiled appearance to the organ.

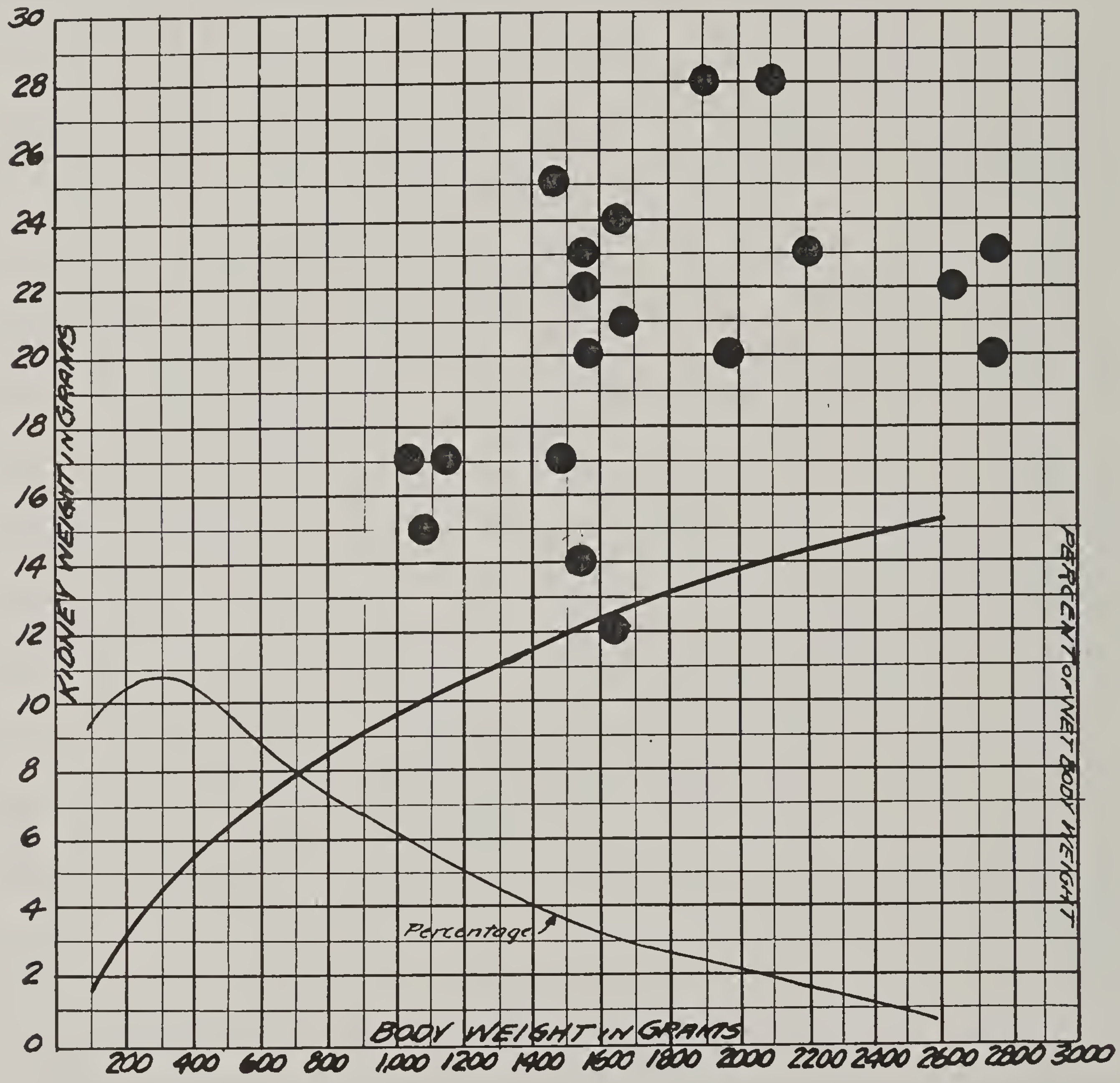

FIG. 24.—GROSS WEIGHT OF KIDNEY IN NORMAL BIRD, AND IN TYPHOID BIRD OF SAME WEIGHT

Liver.—The liver is dark, enlarged, and blood drips from the sectioned surface. Microscopic examination shows the cells in various stages of cloudy swelling and focal necrosis. Figure 26 shows a photomicrograph of a section of the liver, in which is seen at A, a congested vessel; B, a focal area of endothelial cell proliferation; C, a hepatic cell in a state of cloudy swelling or albuminoid degeneration. There may be observed small microscopic areas made up of a mass of small spheroidal cells, due to obstruction of liver capillaries in part by proliferation of endothelium. Necrotic changes may develop in and around these focal cell accumulations. The focal necroses of the liver and of other parenchymatous organs is due to the action of toxic substances in the body fluids as a result of the infection.

Spleen.—Microscopic examination of an enlarged spleen shows it congested or engorged with blood, giving it a dark red appearance on gross examination. There also may be present hyperplasia which further accounts for the enlarged condition. In reference to typhoid in humans, Mallory describes proliferation of endothelial cells, especially in the blood vessels and pulp spaces, and the formation of venus thrombi. This finding corresponds very closely with the microscopic findings in the spleen of a bird dying from avian typhoid.

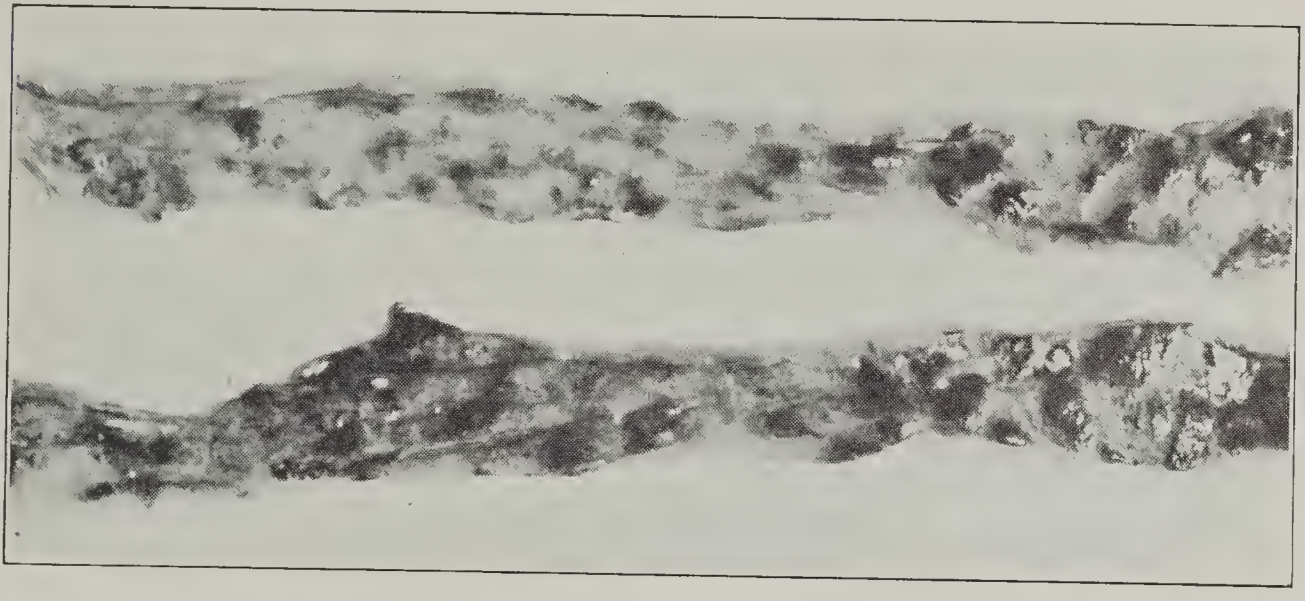

FIG. 25.—SUGGILLATION OF MUCOSA OF SMALL INTESTINE

Kidneys.—The kidneys are the seat of active congestion caused by the toxin produced in this disease. Degenerative changes, such as cloudy swelling, cellular infiltration and areas of focal necrosis are to be found. In Figure 27 is shown a photomicrograph showing active congestion (A); cloudy swelling (B), in which the cells are swollen occluding the lumen of the tubules. C shows two of the tubes in which the cells are leaving their base.

Lungs.—In some cases broncho-pneumonia is present. There is extravasation of serum into the alveoli due to injury of the capillaries by the toxin given off to the body fluids by the infecting organism. Congestion is present. Figure 28 shows a photomicrograph under low magnification showing congestion (A) and some serous effusion (B).

Table VIII

EFFECT OF FOWL TYPHOID ON BODY WEIGHT OF ADULT BIRDS UNDER ARTIFICIAL INFECTION CONDITIONS

WHITE PLYMOUTH ROCK

Pre-Inoculation

Temperature *A. M.*	*P. M.*	*Body Weight*
107.0	106.2	4180 Grams

Sub-Inoculation

A. M.	*P. M.*	*Body Weight*
107.0	106.2	4180 Grams
107.0	106.2	4092 Grams
106.2	105.4	4105 Grams
107.2	106.2	4108 Grams
106.6	106.0	4130 Grams
108.7	109.6	4074 Grams
108.2	108.6	4015 Grams
108.4	109.8	3947 Grams
Bird died; weight dead bird		3947 Grams

RHODE ISLAND RED

Pre-Inoculation

Temperature *A. M.*	*P. M.*	*Body Weight*
106.9	106.6	1880 Grams

Sub-Inoculation

A. M.	*P. M.*	*Body Weight*
107.6	106.6	1864 Grams
107.0	106.0	1790 Grams
107.2	106.6	1897 Grams
107.8	108.6	1828 Grams
109.4	108.5	1771 Grams
109.5	109.6	1731 Grams
109.6	109.8	1580 Grams
109.4	Weight of dead bird	1580 Grams

The temperature on the last morning was taken at 8:00 A. M.; the bird was dead at 8:45.

WHITE PEKIN DUCK

Pre-Inoculation

Temperature	*Body Weight*
107.2	2500 Grams

Sub-Inoculation

Temperature	*Body Weight*
108.2	2189 Grams
107.5	2306 Grams
105.8	2300 Grams
106.2	2320 Grams
108.7	2350 Grams
107.2	2360 Grams
107.4	2390 Grams
107.8	2277 Grams
107.8	2280 Grams

The duck had thrown off the disease after giving definite clinical symptoms on the 5th day.

Table VIII—CONTIUNED

MALE GUINEA

Pre-Inoculation

Temperature	*Body Weight*
107.0	1301 Grams

Sub-Inoculation

108.6	1263 Grams
109.4	1329 Grams
108.8	1205 Grams
108.8	1199 Grams
109.5	1180 Grams
The guinea died; weight of dead bird	1180 Grams

RHODE ISLAND RED

Pre-Inoculation

106.5	3100 Grams

Sub-Inoculation

107.0	3062 Grams
105.0	3063 Grams
106.8	3053 Grams
106.6	3055 Grams
107.5	3041 Grams
109.0	2975 Grams
109.3	2921 Grams
109.0	2800 Grams
110.5	2715 Grams
The bird died; weight	2700 Grams

RHODE ISLAND RED

Pre-Inoculation

Temperature	*Body Weight*
105.8	3100 Grams

Sub-Inoculation

104.5	3100 Grams
106.1	3090 Grams
106.0	3080 Grams
106.6	3060 Grams
105.8	3071 Grams
109.4	3050 Grams
109.0	3021 Grams
109.5	2900 Grams
109.7	2905 Grams
109.2	2885 Grams
108.1	2805 Grams
108.1	2780 Grams
The bird died; body weight	2780 Grams

Table VIII—Continued

WHITE LEGHORN

Pre-Inoculation

Temperature	*Body Weight*
106.6	1610 Grams
107.4	1592 Grams

Sub-Inoculation

Temperature	*Body Weight*
107.0	1655 Grams
106.8	1690 Grams
106.6	1610 Grams
108.4	1575 Grams
113.0	1530 Grams
Weight of dead bird	1530 Grams

WHITE LEGHORN

Pre-Inoculation

Temperature	*Body Weight*
107.0	1419 Grams
107.0	1402 Grams

Sub-Inoculation

Temperature	*Body Weight*
107.4	1316 Grams
106.9	1402 Grams
106.9	
109.8	1395 Grams
108.8	1298 Grams
108.2	
108.7	1272 Grams
108.6	1261 Grams
109.7	1211 Grams
109.2	1196 Grams
108.6	1221 Grams
109.4	1212 Grams
107.2	1297 Grams
108.2	1287 Grams
108.2	1287 Grams
107.8	1337 Grams
108.4	1345 Grams
106.4	1482 Grams
108.2	
107.5	1434 Grams
107.8	1492 Grams
107.5	1460 Grams
107.0	1450 Grams

The bird recovered.

THE BLOOD DURING DISEASE

In the normal blood of the fowl there is from 3,000,000 to 4,000,000 erythrocytes (12), with an average of 3,500,000 and from 45,000 to 55,000 thrombocytes. There are from 28,000 to 35,000 leucocytes per cu. mm. The leucocytes are divided into five distinct groups: Polymorphonuclear leucocytes with eosinophilic rods, and polymorphonuclear leucocytes with granules; lymphocytes, numbering from 40 to 44 per cent; large mononuclear cells numbering from 18 to 20 per cent, and mast cells or basophiles numbering from 2 to 4 per cent. The per cent hemoglobin appears low in the fowl, being placed at 76 to 88 per cent (29).

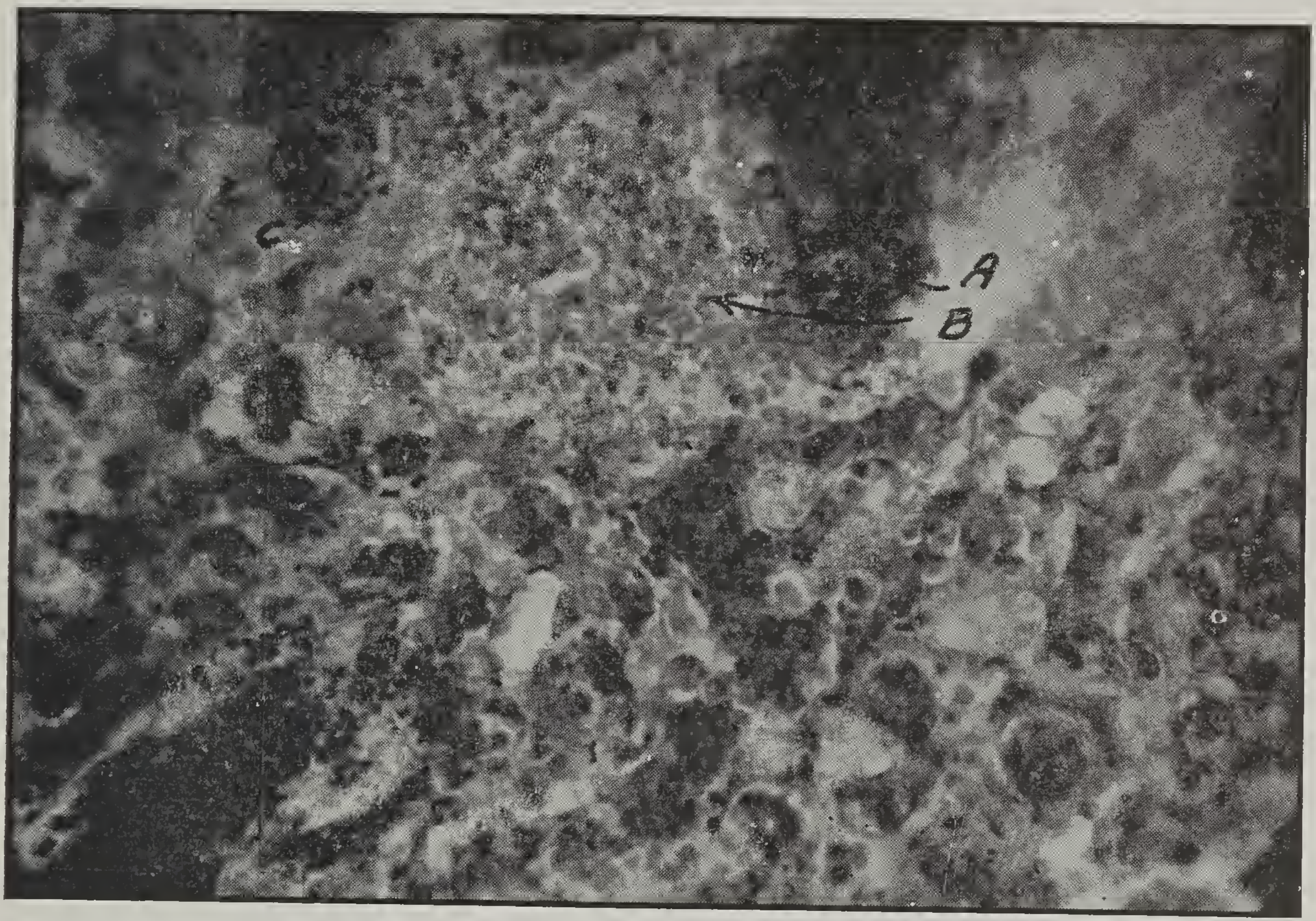

FIG. 26.—PHOTOMICROGRAPH OF SECTION OF TYPHOID LIVER. A. CONGESTION OF LIVER. B. FOCAL AREA OF ENDOTHELIAL CELL PROLIFERATION. C. HEPATIC CELL IN STATE OF CLOUDY SWELLING

Taylor (30) reports in Fowl Typhoid a reduction in erythrocytes and a high increase in leucocytes, especially of the polymorphonuclear variety. This report is supported by the earlier work of Moore (23).

In Table IX will be seen a complete study of three representative cases taken from artificial infection work conducted during this investigation. These studies show a consistent increase in the polymorphonuclear leucocytes. The polymorphonuclear leucocytes with eosinophilic granules shown by the table to be dominant before inoculation, are replaced with cells showing eosinophilic rods after the disease is established. Many degenerated leucocytes, or so-called "basket cells" (34) are observed after the disease is well developed. In chronic cases the destruction of the erythrocytes is less marked and there is less degre eof leucocytosis. There is an increase of thrombocytes, and a decrease of hemoglobin. The coagulability of the blood which normally in the fowl is brought about in thirty seconds is greatly lengthened, and finally in cases where severe changes have taken place, the blood appears quite serous. DeCosta (6) reports a marked lack of coagulability of the blood in human typhoid in early stages. He also reports decrease in the hemaglobin content, a marked reduction of erythrocytes, and a leucopenia corresponding to the severity of the disease. The hematological picture of avian typhoid and human typhoid is similar, except in the case of the numerical count of the leucocytes, in which in fowls there is a leucocytosis

Table IX

COMPOSITE TABLE SHOWING BLOOD STUDIES OF BIRDS INFECTED WITH E. SANGUINARIA

WHITE PLYMOUTH ROCK HEN

Date	Temperature		Body Weight, Grams	Hemoglobin	Numerical Count			Differential Count							Remarks
								Eosinophiles			Lymphocytes				
	A.M.	P.M.			Red Cells	White Cells	Thrombocytes	Rods	Granules	Basophiles	Large	Small	Mononuclears	Transitionals	
Feb. 20	107.0	106.2	4180	85	3,320,000	28,000	90,000	0	65	0	3	26	2	2	Pre-inoculation; bird normal
21	107.0	106.2	4180	85	3,500,000	35,000	79,000	2	57	2	5	31	3	0	Pre-inoculation; bird normal
22	107.0	106.2	4092	85	3,610,000	35,000	77,000	4	62	2	4	27	0	1	Day of inoculation; bird normal
23	106.2	105.4	4105	85	3,200,000	42,000	90,000	1	61	1	7	30	0	0	Bird normal
24	107.2	106.2	4108	80	2,420,000	35,000	120,000	0	60	1	8	31	0	0	Bird normal
25	106.6	106.0	4130	85	2,500,000	46,000	270,000	2	50	1	13	31	1	2	Bird depressed
26	108.7	109.6	4074	80	950,000	56,000	410,000	47	14	0	5	34	0	0	Bird depressed; sulphur-colored discharge
27	108.2	108.6	4015	75	1,750,000	43,000	210,000	10	67	4	1	15	0	0	Bird in semi-conscious condition
28	108.4	109.8	3947	70	1,430,000	37,000	180,000	1	75	2	1	10	7	4	Died in convulsions

Bird died.

WHITE PEKIN DUCK

Date	A.M.	P.M.	Body Weight, Grams	Hemoglobin	Red Cells	White Cells	Thrombocytes	Rods	Granules	Basophiles	Large	Small	Mononuclears	Transitionals	Remarks
Mar. 31	107.2	--------	2500	85	2,960,000	35,000	--------	1	40	1	8	50	------	------	Pre-inoculation; duck normal
April 1	107.2	--------	2189	85	--------	--------	--------	------	------	------	------	------	------	------	Pre-inoculation; duck normal
2	107.4	--------	2306	85	3,200,000	35,000	--------	7	45	------	7	41	------	------	Day of inoculation; duck normal
3	105.8	--------	2300	80	3,400,000	23,000	--------	48	3	2	5	42	------	------	Duck normal
4	106.2	--------	2320	75	3,100,000	28,000	--------	------	------	------	------	------	------	------	Duck normal
5	108.7	--------	2350	70	3,200,000	27,000	--------	17	30	1	2	45	1	4	Duck normal
6	107.2	--------	2360	65	3,160,000	31,000	--------	------	------	------	------	------	------	------	Depressed; sulphur-colored discharge
7	107.4	--------	2390	70	2,900,000	38,000	--------	9	45	1	4	40	------	------	Duck normal
8	107.8	--------	2277	70	2,830,000	48,000	--------	------	56	------	4	40	------	------	Duck normal
9	107.8	--------	2280	85	3,100,000	37,000	--------	------	------	------	------	------	------	------	Duck normal

Duck recovered.

PEARL GUINEA (MALE)

Date	A.M.	P.M.	Body Weight, Grams	Hemoglobin	Red Cells	White Cells	Thrombocytes	Rods	Granules	Basophiles	Large	Small	Mononuclears	Transitionals	Remarks
April 6	107.0	--------	1301	95	3,360,000	23,000	--------	4	86	------	2	7	------	------	Pre-inoculation; bird normal
7	107.6	--------	1263	90	3,500,000	25.000	--------	7	75	1	4	13	------	------	Day of inoculation; bird normal
8	109.4	--------	1329	95	3,530,000	27,000	--------	4	80	3	1	8	1	3	Sulphur-colored discharge; bird depressed
9	108.8	--------	1205	90	3,000,000	42.000	--------	11	65	1	5	15	------	3	Sulphur-colored discharge; bird depressed
10	108.8	--------	1199	80	2,500,000	50,000	--------	12	69	2	4	12	------	1	Sulphur-colored discharge; bird depressed
11	109.5	--------	1180	80	1,000,000	70,000	--------	4	75	------	7	14	------	------	Sulphur-colored discharge; bird depressed

Guinea died.

CONTROL MEASURES

The ultimate aim in experimental work in the prevention and control of contagious disease is to eliminate the sources and conveyors of the contagium, and in the establishment of such immunizing processes as will build up the immunity of the possible host to such an extent as will enable it to throw off the effects of bacterial invasion.

Sanitary conditions noted in the investigations in the field during these studies could not be rated as better than fair. In most instances the housing was poor; the birds were on free range; no germicide was used in the drinking water of the infected flocks to prevent the dissemination of the disease in this manner, and although sick birds were isolated, and carcasses safely disposed of, conditions in general favored the inception and spread of disease. In most of the outbreaks investigated the disease had assumed well defined proportions before the owner was sufficiently impressed with its seriousness to seek assistance. This fact accounts for the heavy infection of houses and premises before remedial steps could be taken to stop the epizootic. As noted before, poor sanitation was undoubtedly responsible for the dissemination of the disease in the flock after the initial inception. In some of the outbreaks investigated the floors and dropping boards had not been cleaned for weeks; the food troughs were bespattered with fecal material of long standing, and the drinking vessels were clotted with filth and sediment; some of the houses were so built as to obtain no direct rays of the sun, and with no provision for ventilation. The birds in many cases were of low vitality, suffering severely from scabies, internal and external parasites, and the general physical condition of the flocks favored the rapid dissemination of the disease.

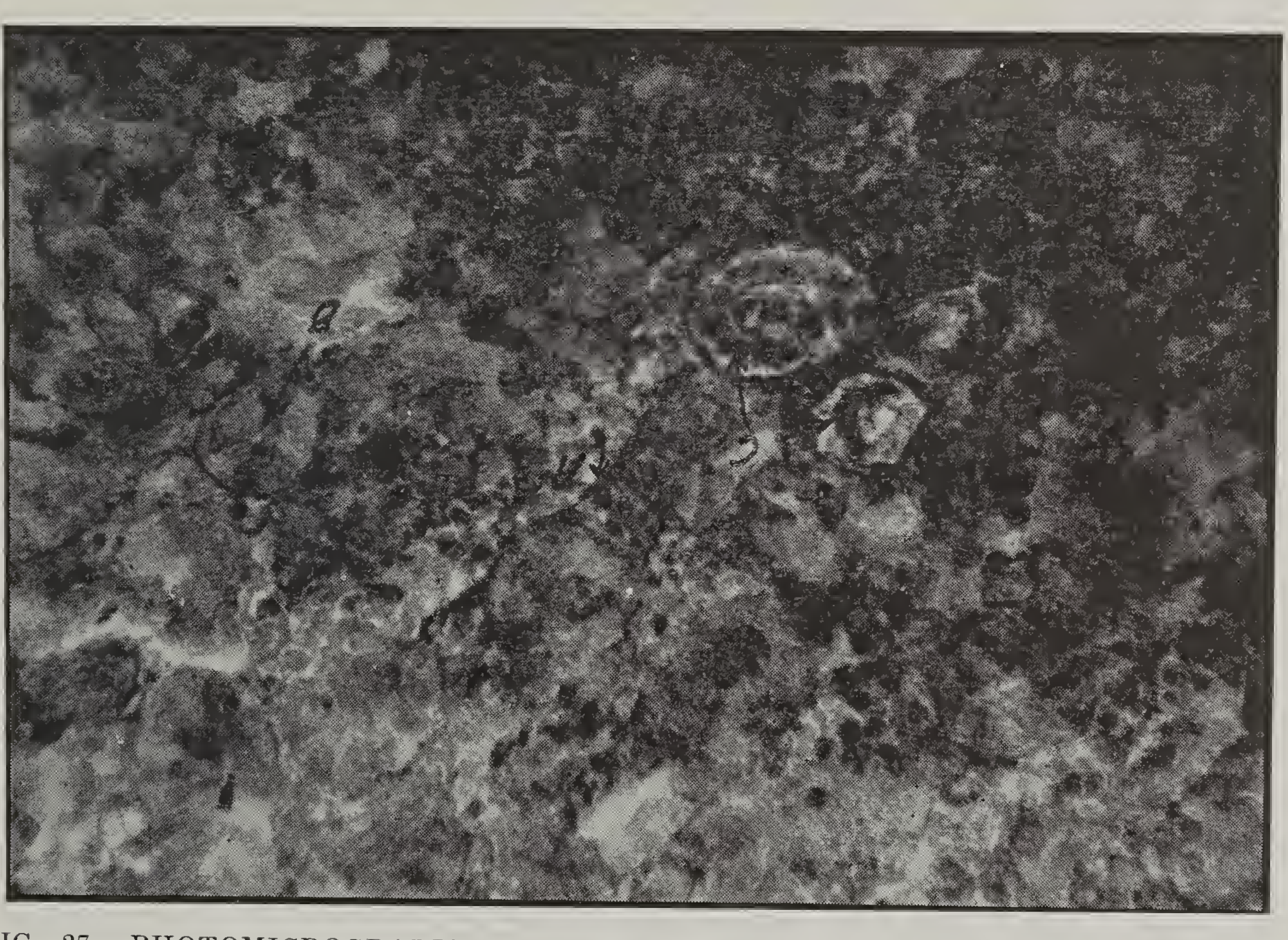

FIG. 27.—PHOTOMICROGRAPH OF A TYPHOID KIDNEY. A. CLOUDY SWELLING. B. SWOLLEN CELLS OCCLUDING THE LUMEN OF TUBULE. C. CELLS LEAVING BASE

The immediate steps taken to break up the spread of the disease was to emphasize strict sanitation, isolation or destruction of sick birds, disinfection of drinking water, confinement of birds to new range grounds if possible, and the vaccination of all well stock. Splendid coöperation was afforded the department by the County Agents and Agricultural High School teachers in outbreaks which could not be reached from the Poultry Pathological laboratories.

The first comprehensive work in vaccination was that of Jenner in 1796. Jenner succeeded in producing immunity against smallpox by using cowpox virus, but was unable to scientifically establish the body processes by which immunity was accomplished. Pasteur in 1879, in his studies with the organism of Fowl Cholera, brought out the important possibility that the virulence of a virus or germ might be so attenuated that its administration is not followed by the actual disease, but by the stimulation of body cells to the production of scientific antibodies. Since the work of Pasteur, serology and vaccine therapy has been the subject of exhaustive investigation and research.

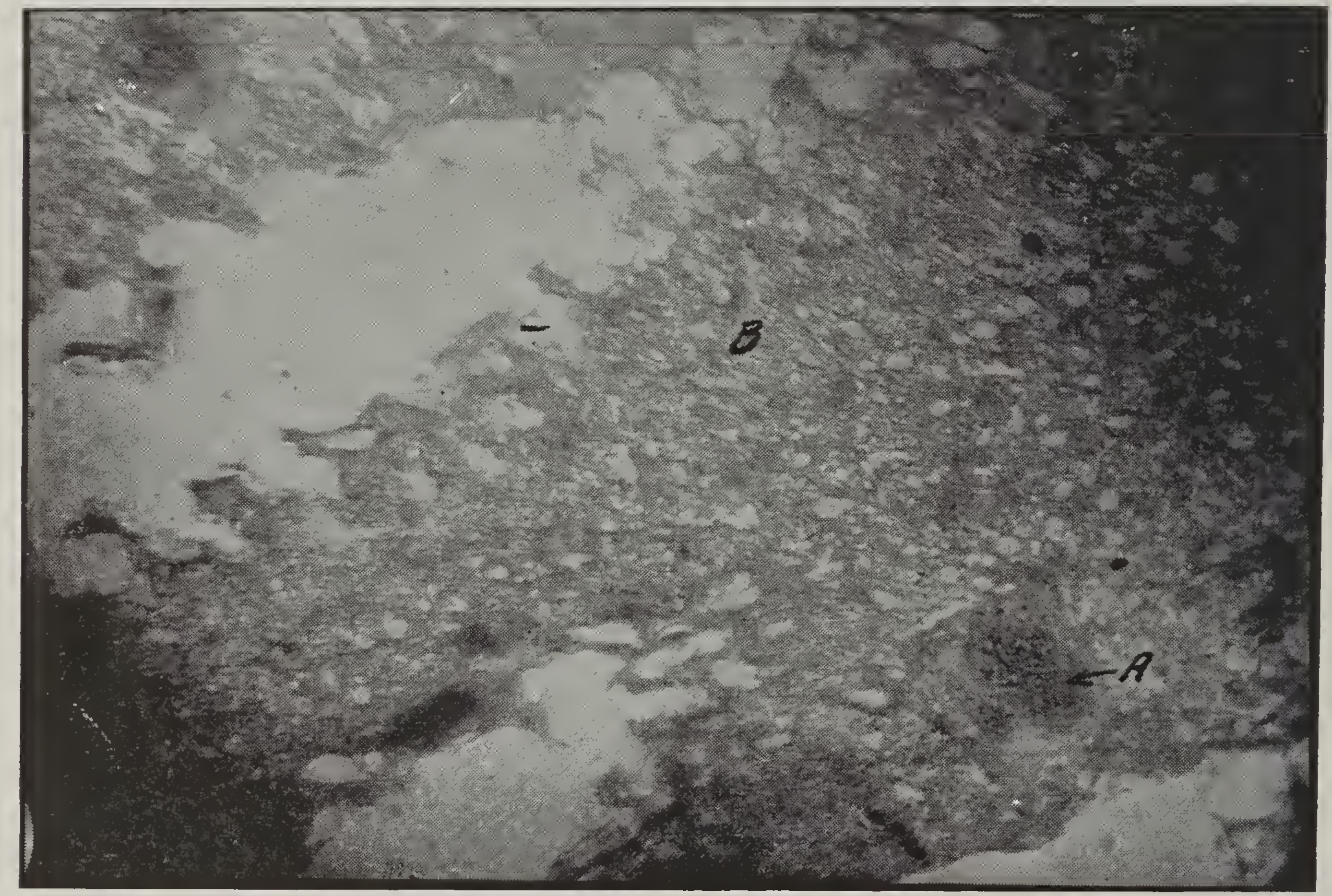

FIG. 28.—PHOTOMICROGRAPH OF A SECTION OF A TYPHOID LUNG.
A. CONGESTION OF BLOOD VESSEL. B. SEROUS EFFUSION

Kolmer (19) defines a vaccine as a "sterilized and enumerated suspension of bacteria which furnish, when they dissolve in the body, substances which stimulate the tissues to a production of specific bacteriotrophic substances, which fasten upon and directly or indirectly contribute to the corresponding bacteria (Wright)."

The use of vaccines in poultry husbandry is limited to a certain degree. Such work, to be useful, must necessarily be placed on an ultra-practical basis. Owing to the small value of the individual bird, and the relative short span of life, the general use of vaccines is not favored by the flock owner. The vaccine must be readily available, and of a low cost to secure usage in disease outbreaks.

Kolmer brings out the following ways in which bacteria or their products might be treated in order to be used for immunizing purposes:

1. The living organism may be inoculated.
2. By inoculation with a modified virus, or with microorganisms attenuated or modified according to various methods.
    a. By passing through a lower animal.
    b. By exposing microorganisms to air and light.
    c. By exposing microorganisms to heat.
    d. By dessicating or drying virus.
    e. By exposing microorganisms to a high temperature for varying lengths of time.
    f. By exposing microorganisms or their products to certain chemicals.
3. By inoculating the bacterial constituents, as soluble toxins, bacterial extracts and products of bacterial extracts.

Bushnell and Patton (2) recognize three conditions in relation to the struggle between the host and invading parasite:

1. In which the host possesses sufficient immunity to rapidly localize and destroy the invading organism.
2. In which the invasion powers of the organism and the reactive powers of the host are more nearly equalized, and the infection becomes localized and perhaps chronic.
3. An entire lack of ability of the host to localize and destroy the invading organisms.

All three of the above conditions were encountered both in the field and in the artificial infection studies on avian typhoid.

The production of antibodies is the basis for prophylactic vaccination (Figure 29.) When antigenic substances, which probably are colloids of a protein nature, are introduced into the body, the antibodies are usually highly specific for the antigen injected, but also may act as a general stimulant, and may induce the body cells to cast off small amounts of the antibody unrelated to the nonspecific agent injected. For disease diagnosis, birds are received at this station in all stages of paralysis. It has been a routine procedure to vaccinate such birds with stock vaccine for avian typhoid, and in some instances noteworthy results have been obtained, a small per cent of such birds responding quickly to this vaccination, and regaining normal coördination of body parts, the results possibly being due to body stimulation produced by the vaccine. It is also worthy to note that attempts to reproduce paralysis were not successful though inoculations were made with the Berkfield filtrate of emulsions of the brain, spinal cord, and various internal organs.

In vaccination in the field against fowl typhoid, vaccines prepared by heating cultures as described by Kolmer in 2c. were used. The objective was the production of artificially acquired immunity for as great a length of time as possible. Autogenous vaccines were used as often as practicable in order that the specific organism might retain such natural potencies, as near as possible, as were maintained in the original organism. The rapid loss of virulence in stock cultures of E. sanguinaria gives rise to the belief that the natural potencies of the organism are changed by adverse conditions and by artificial life. Variants are also possible among the specie which might delay the processes of immunization if stock vaccines were used, and from field observations it was apparent that the mortality was checked with greater rapidity where the autogenous vaccine was employed.

The use of heated suspensions proved the most practical method of making avian typhoid vaccines, and the most economical method of meeting the heavy demand made on the laboratory, although the antigenic powers of a vaccine prepared in this way are inferior to the living antigen because of the principles which might be lost through the heating process.

The quantity of vaccine given was 1 cc. per bird during the early part of this work. This was due to the fact that it was deemed expedient to develop single vaccination if possible, because of the reluctance of the poultryman to submit his flock to double or triple vaccination. The number of organisms used was approximately 1,000,000,000 per cc. Later the dosage was increased to 2 cc. to the adult bird and one-half cc. to the younger ones. The field studies show that usually one vaccination was sufficient to immunize, only in three outbreaks did serious recurrence occur.

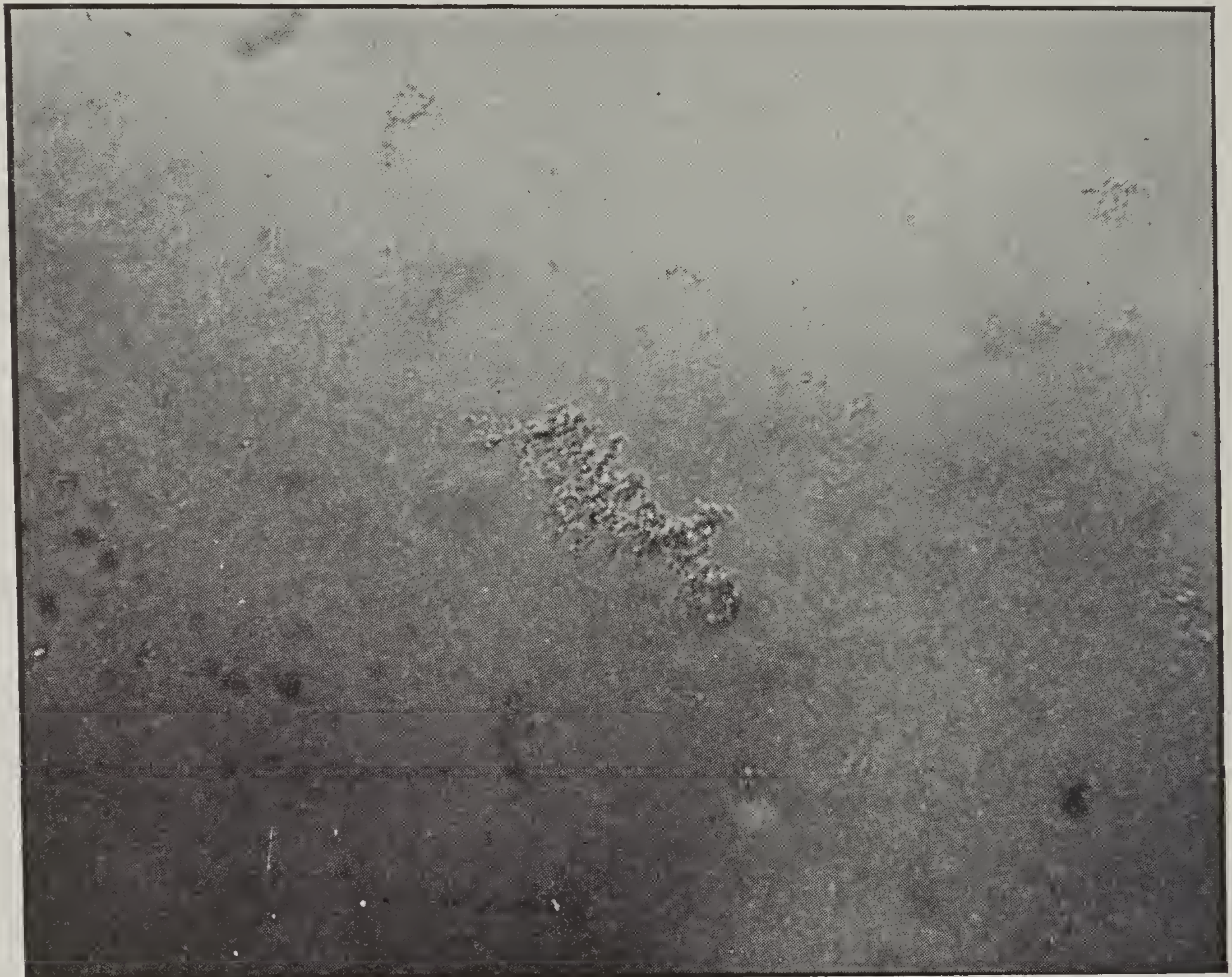

FIG. 29.—PHOTOMICROGRAPH OF WIDAL TEST. REACTION OCCURRING FIVE DAYS AFTER INFECTION

The duration of immunity produced by prophylactic vaccination against avian typhoid is not definitely known. This duration will probably vary greatly according to the reaction of the individual to the vaccine, and to the antigenic properties of the same. Bushnell and Patton (2) after examining 100 vaccinated birds conclude that the duration of immunity is not over 60 days. In the case of the single inoculation, this department carried on a series of adult leghorns that had received 1 cc. of avian typhoid vaccine for six months, attempting to infect one of these birds each month. None of these birds contracted the disease. Two birds which had been vaccinated three years prior to the artificial studies were readily infected with avian typhoid and succumbed to the disease. Studies of the data in the mortality table showing the reports on vaccination involving outbreaks during the past three years seems to indicate that the single inoculation has proved efficient in the great majority of instances. Follow-up visits to these outbreaks indicate that the disease has been efficiently checked, although it is premature to make any general assertion as to the possibilities of recurrence through the medium of soil pollution or carriers.

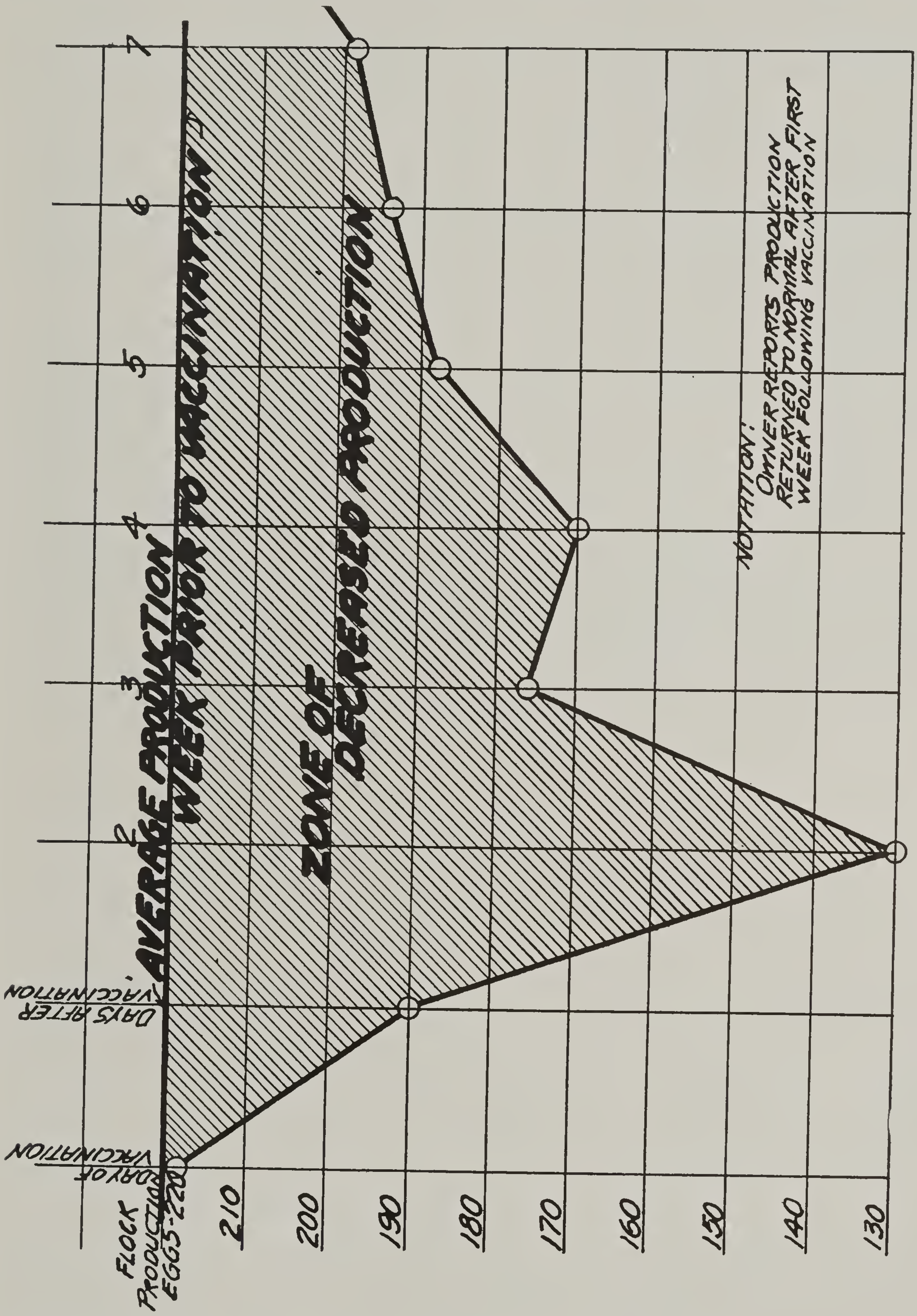

FIG. 30.—THE EFFECTS OF VACCINATION ON EGG PRODUCTION

The phenomena of cross-agglutination between members of the colon-typhoid intermediates has presented an interesting phase to this specific group of organisms. The data regarding cross-agglutination as worked out in this laboratory is presented in Table II, in the study of the causative organism. The findings is in agreement with other investigators of this phenomena.

d'Herelle (4) has reported the presence of a bacteriophage against avian typhoid in his experimental work on this phenomena as recorded in "The Bacteriophage." The role of the bacteriophage as a prophylactic agent under natural conditions is problematical, but it is highly probable that d'Herelle's principle is active in some of the birds that resist the disease. A bacteriophage was successfully recovered in two instances in the artificial infection work in this laboratory from the fecal material of birds which threw off the disease. The virulence of the bacteriophage isolated was two plus against E. sanguinaria.

Vaccination of birds with avian typhoid vaccine failed to demonstrate an upset of body temperature, though a depressing effect was sometimes noticed in the field. In the matter of egg production, there was manifested a decided decline in production for seven days after vaccination, the decline being greatest the first three days. Normal production was resumed in from 7 to 10 days, as shown in Figure 30.

The possibility of immunity being established in the domestic fowl against avian typhoid by the use of vaccines prepared for human usage with the Bacillus typhosus has been considered, but in an experiment carried out in conjunction with this work such immunity could not be demonstrated. Two adult birds were vaccinated with the vaccine for humans, one receiving one vaccination of 1 cc. and the second bird two vaccinations 7 days apart. Both of these birds succumbed from infection with avian typhoid, and the causative organism was recovered. A similar attempt was made to immunize with the triplex vaccine for humans. The bird receiving the single vaccination succumbed to infection with E. sanguinaria, the bird receiving the double vaccination contracted the disease, but recovered.

FOWL CHOLERA

Historical.—The disease known as Fowl Cholera is endemic in some sections of the world. Referring to this disease, Hutyra and Marke (11) give the following historical resume:

"This dangerous disease was described by veterinarians as early as the 18th century, and in the middle of the last century some of the authors (Benjamin, 1851; Delafond and Renault, 1851; and Hering, 1858) have recognized its contagious nature, and also substantiated it experimentally. Perroncito (1878) and also Semmer, described a diplococcus as the cause of the disease, which they constantly found in the blood of chickens dead of the disease. Toussant (1879), and later Pasteur (1880), confirmed their findings and succeeded in growing the bacteria in artificial bouillon, thus enabling them to study the pathogenic characteristics. Pasteur carried out his first fundamental experiments on the attenuation of bacterial cultures, and on immunization with such attenuated cultures with the bacilli of fowl cholera. Valuable contributions regarding the etiology of the disease were also furnished by Kitt and Lignieres."

In America, the disease was first reported and studied by Salmon (28) in 1880. Salmon summarizes as follows:

"If we examine the reports received each year at this Department from the different parts of the country we find that chicken cholera is mentioned as being destructive to the fowls in more than half the counties heard from. The losses are estimated all the way from a few hundred dollars to as high as two hundred thousand dollars in single counties and if we consider that the remaining counties are affected to an equal extent, it is not difficult to realize the immense amount of capital that is annually swept out of existence by this plague. It may be that ten millions of dollars would cover the annual loss, but it is about as likely to reach fifteen or twenty millions."

In view of the fact that Salmon's investigations were prior to the work of Klein in England (1888) and of Moore in America (1895) on fowl typhoid, it is possible that a good part of the disease designated as cholera in this survey was infection with E. sanguinaria (fowl typhoid). Cholera later was subjected to rigorous investigation by various American scientists, the work of Hadley being outstanding.

Distribution.—The distribution of avian cholera and of avian typhoid is general throughout the world where poultry is raised. Undoubtedly intensive poultry raising and poor sanitary conditions are conducive to the inception and spread of these diseases. In Europe, both diseases are prevalent at the present time. Dr. B. J. C. teHennepe of the State Serum Laboratories, Rotterdam, sums up the situation on the Continent in a paper before the World's Poultry Congress at The Hague in September, 1921, in the following statement:

"The number of cases or outbreaks observed by the State Serum Laboratories at Rotterdam, from 1905 to 1920, were: cholera, 101; typhoid, 463. The above-mentioned figures prove clearly that in The Netherlands the Klein (typhoid) disease is of much greater importance than cholera. This opinion is shared as far as France is concerned by F. d'Herelle. Also, in that country, cholera is less frequent than typhoid."

d'Herelle's statement about these two diseases in France is as follows:

"Avian typhosis is a disease affecting primarily the gallinaceae. Despite its frequency it for a long time remained undetected, confounded with chicken cholera. This disease is, in reality, very rare."

In America, special stress seems to be laid on fowl cholera in the West and Middle West, where exhaustive studies, especially on the serological side of the question, have been conducted by several experiment stations. In the northeastern section, both the diseases are reported to a considerable extent, while in the South, the avian typhoid seems to be more prevalent than cholera. In North Carolina, 55 outbreaks have been diagnosed as fowl typhoid in the last two years, with no outbreaks attributed to the fowl cholera pasteurella.

The Causative Organism.—The causative organism of avian cholera is designated as the Pasteurella avicida according to the Classification of The Society of American Bacteriologists, 1917-20.

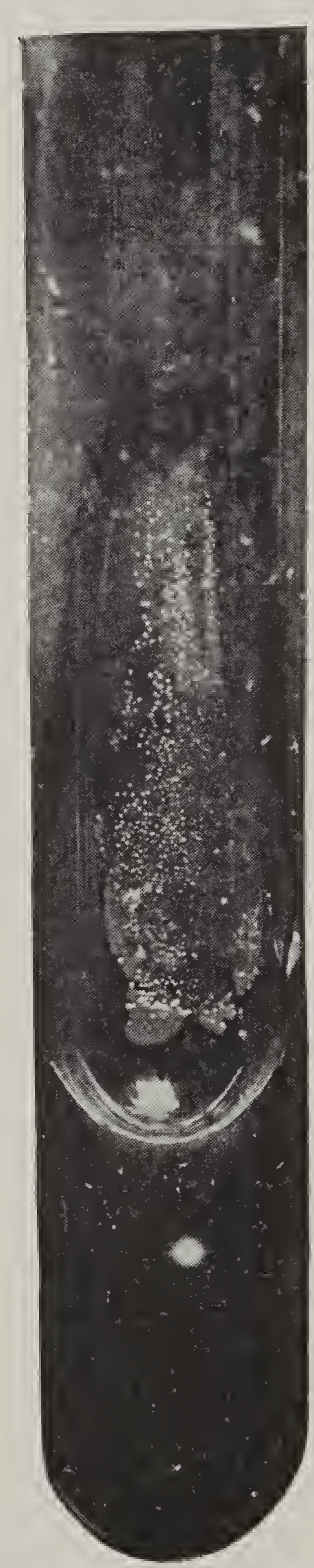

FIG. 31.—P. AVICIDA ON GLYGERINE-AGAR SLANT

MORPHOLOGICAL STUDIES

The Pasteurella avicida is a nonmotile, gram negative organism showing no capsule, endo spores or irregular forms. The size of the organism varies, the average being 0.3 by 0.5 microns. The ends are rounded, the organism stains bi-polar, which tendency is sometimes lost on prolonged cultivation in artificial medium.

Cultural Characteristics

Agar Slant (Glycerine).—(Fig. 31.) The growth is scanty; echinulate; raised; smooth; glistening, and gives no odor.

Agar Colonies (Glycerine).—P. avicida makes rapid growth on agar, the colonies being punctiform, smooth, raised, entire, and of mucid consistency.

Gelatin Stab.—There is a translucent uniform growth; non-liquifying, with the medium remaining unchanged.

Potato.—There is no growth on potato.

Nutrient Broth.—There is no surface growth; slight turpidity; no odor; heavy viscid sediment in 5 days.

Temperature Relations.—The optimum temperature for growth is 37½ degrees C., with slight tolerance to extremes of heat or cold.

Reaction to Medium.—Luxuriant growth is obtained on 3% neutral glycerine agar; P. avicida has little tolerance to organic acids.

Production of Indole.—Indole is formed with the Salkowsli test; slight formation with the vanilin test, and with the Ehrlich test in some instances. These reactions are not consistent.

Chromogenisis.—The organism is non-chromogenic.

Relation to Free Oxygen.—Slight aerobic growth on glucose-agar shake in 24 hours.

Milk.—Milk is unchanged.

Nitrate Reduction.—Nitrates are reduced.

Hydrolysis of Starch.—Slight diastatic reduction on starch agar.

FERMENTATION STUDIES

Table X

Reactions Produced by P. Avicida in Carbohydrates and Higher Alcohols

Carbohydrate or Higher Alcohol	*Number of Strains*	*Gas Production*	*Orig. pH.*	*pH after 5 days (Average)*	*Reaction*
Arabinose	10	0	7.0	7.0	Neutral
Dextrin	11	0	7.0	7.0	Neutral
Dextrose	19	0	7.0	6.0	Acid
Dulcite	11	0	7.0	7.0	Neutral
Galactose	11	0	7.0	7.0	Neutral
Inulin	11	0	7.0	7.0	Neutral
Lactose	14	0	7.0	7.0	Neutral
Levulose	11	0	7.0	7.0	Neutral
Mannose	11	0	7.0	7.0	Neutral
Maltose	12	0	7.0	7.0	Neutral
Saccharose	14	0	7.0	7.0	Neutral
Xylose	11	0	7.0	7.0	Neutral

Pathogenicity

Non-pathogenic to man. Virulent strains of P. avicida will produce death in 12 to 18 hours in mice, and the same with rabbits.

Symptoms of the Disease

The study of avian cholera was made under artificial infection conditions. Thirty-five artificial infections were made with cultures from American and from European sources. Ten fatalities were obtained, seven showed well-defined symptoms of the disease, and eighteen birds failed to react to artificial infection. The rapid attenuation of the cholera cultures on artificial media was undoubtedly responsible for a lack of virulence in some of these instances. The incubation period was from one to three days, one bird dying overnight with the disease. The acute type of fowl cholera is very rapid, the duration in fatal cases averaging less than five days. One bird resisted the disease for fourteen days before succumbing. Depression is usually the first symptom; the discharge is profuse, being flaked with red particles in the early stages. Later, the discharge becomes reddish green, and very watery. The face, comb, and wattles usually become darkened and discolored. An exceptional thirst is very characteristic of the disease. Respiration is increased, and later becomes labored; there is an elevation in temperature; complete prostration sets in preceding death.

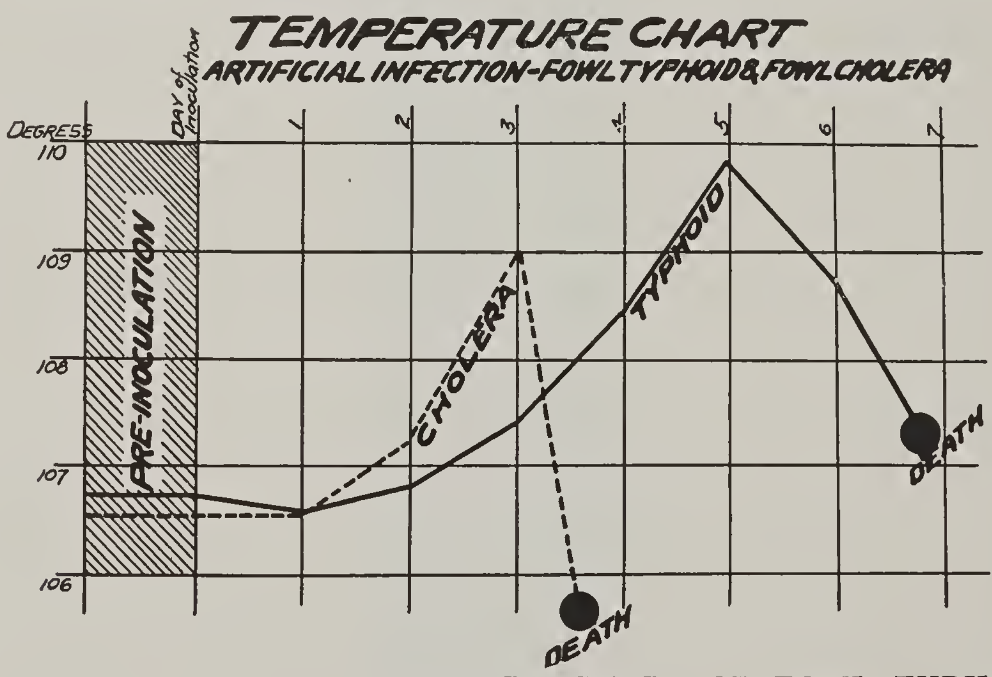

FIG. 32.—A COMPOSITE TEMPERATURE CHART OF FOWL TYPHOID AND FOWL CHOLERA

Figure 32 shows a composite temperature chart of the diseases of fowl typhoid and fowl cholera.

ANATOMICAL CHANGES

Gross Anatomy

In acute cases, body emaciation is not apparent. A cyanotic condition of the face, comb and wattles is apparent. In some instances there is a darkening of the breast muscles. The coronary band of the heart is studded with petechial hemorrhages. The organ is slightly enlarged. The liver is usually spotted with areas of parenchymatous degeneration, and is slightly enlarged. The lungs are in a serofibrinous condition, sometimes approaching the pneumonic stage. The spleen may or may not be enlarged. The kidneys are slightly swollen. The intestines show an acute hemorrhagic inflammation, and the intestinal contents are intermingled with a bloody mucous material.

Microscopic Anatomy

Heart.—There is slight congestion, with a tendency for the muscle fibers to loose their cross-striation.

Liver.—The liver shows both active and passive congestion.

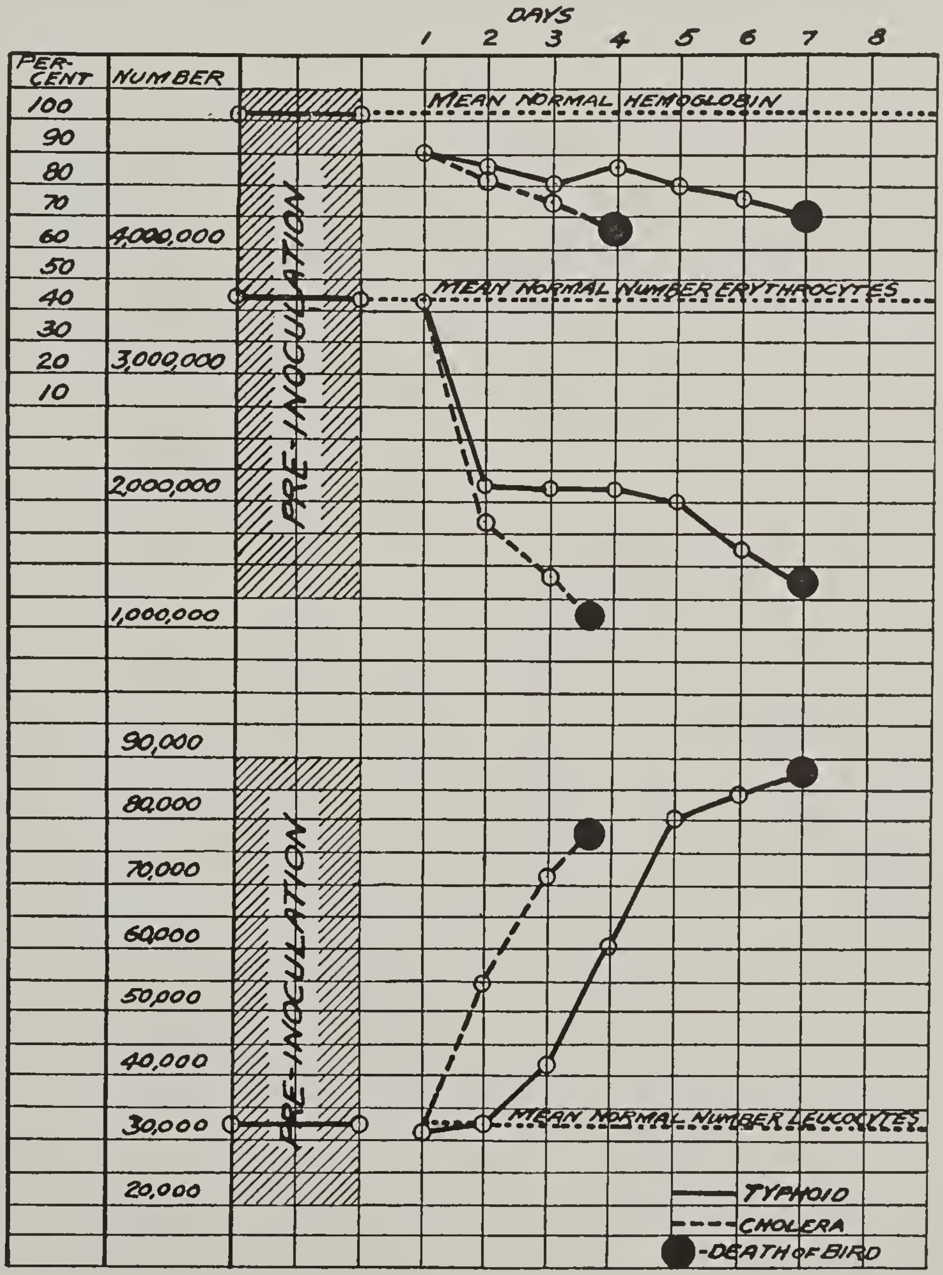

FIG. 33.—A COMPOSITE GRAPH OF BLOOD IN FOWL TYPHOID AND FOWL CHOLERA

Kidneys.—Cloudy swelling and areas of focal necrosis are present; some cells are in a state of pyknosis; in some areas, the cells leave their base, passing to the center, obliterating the lumen; in other areas, the nuclei and cells are in a state of disintegration; active and passive congestion are present.

Hematological Studies

There is a relative decrease in the number of erythrocytes with an absolute leucocytosis ensuing. A differential cell-count shows the lymphocytes running as low as 5 per cent and the polymorphonuclear leucocytes as high as 95 per cent. There is a decided lack of coagulability of the blood, and the hemoglobin is reduced.

Figure 33 shows a composite graph of the blood in the diseases of avian typhoid and avian cholera.

Artificial Infection Studies

Table XI shows the results of artificial infection with P. avicida.

Table XI

Clinical Studies of Artificial Infected Birds

Breed	Sex	Strain Used	Infected per—	First Symptom, Days	Duration to Death, Days	Duration to Recovery Days	No Symptoms, Days	Temperature		Respiration	
								Max.	Min.	Max.	Min.
B. P. Rock	Ckl	American	Orum	3	14	------	------	108.7	105.7	41	18
R. I. Red	F	American	Orum	5	------	15	------	108.6	103.3	42	31
W. Leghorn	F	French	Orum	3	8	------	------	109.4	103.6	37	21
W. Leghorn	F	English	Orum	------	------	------	11	108.0	105.2	64	56
R. I. Red	F	American	Orum	------	------	------	11	107.5	106.0	48	36
W. Leghorn	Ckl	English	Drinking water	3	------	9	------	109.0	106.0	------	------
W. Leghorn	Ckl	American	Orum	2	------	9	------	108.7	105.8	54	40
W. Leghorn	F	------------	Fecal matter from No. 3 in drinking wtr.	------	------	------	7	107.0	106.0	35	22
W. Leghorn	Ckl	American	Orum	------	------	------	10	107.8	106.4	56	42
W. Leghorn	F	English	Orum	2	------	9	------	108.8	106.8	------	------
J. B. Giant	F	English	Orum	2	------	11	------	108.2	104.8	27	24
B. P. Rock	F	Holland	Orum	2	4	------	------	109.1	106.8	30	26
R. I. Red	F	Holland	Orum	2	3	------	------	109.0	106.4	32	28
R. I. Red	F	American	Orum	3	7	------	------	109.6	106.0	32	24
R. I. Red	Ckl	American	Orum	1	1	------	------	107.0	106.1	30	22
R. I. Red	F	American	Orum	3	10	------	------	109.0	105.0	32	17
R. I. Red	F	American	Orum	5	11	------	------	111.2	107.0	30	19
B. P. Rock	F	American	Orum	3	13	------	------	110.5	107.5	42	16
B. P. Rock	F	American	Orum	4	7	------	------	111.0	106.5	36	31
Mongrel	F	American	Orum	------	------	------	7	106.4	105.0	40	30
Mongrel	F	American	Orum	------	------	------	7	107.7	105.8	34	28
Mongrel	F	American	Orum	------	------	------	11	107.1	106.0	34	28
Mongrel	F	American	Orum	------	------	------	7	106.6	105.0	40	32
Mongrel	F	American	Orum	------	------	------	11	107.0	106.0	32	23
Mongrel	F	American	Orum	------	------	------	11	107.4	105.7	26	20
Mongrel	F	English	Orum	------	------	------	5	106.7	105.7	35	24
Mongrel	F	French	Orum	------	------	------	5	107.0	106.2	37	28
B. P. Rock	F	Hungarian	Orum	5	------	13	------	109.2	106.2	38	29
Buff	F	Hungarian	Orum	2	------	11	------	109.6	106.4	38	24
Mongrel	F	American	Orum	------	------	------	7	107.5	106.2	42	26
Mongrel	F	American	Orum	------	------	------	7	107.8	106.8	38	28
Mongrel	F	American	Orum	------	------	------	7	106.8	105.8	40	28
Mongrel	F	American	Orum	------	------	------	7	107.0	105.9	42	32
Mongrel	F	American	Orum	------	------	------	7	107.4	106.5	36	32
Mongrel	F	American	Orum	------	------	------	7	107.2	106.2	38	28

SUMMARY

FOWL TYPHOID

1. Avian typhoid is a particularly virulent contagium attacking principally the domestic fowl. The causative agent is of bacterial origin—Eberthella sanguinaria (Moore).

2. The disease is probably prevalent in all countries where poultry husbandry is practiced. It is widely distributed in North Carolina where it occurs during the late fall, winter and early spring.

3. The disease has been the subject of investigation as Avian Typhoid since 1888. Investigations prior to that time were made under the assumption that the disease was true cholera.

4. Avian typhoid is non-pathogenic to man. Chickens are very susceptible to the disease; turkeys and guineas are less susceptible; pigeons, ducks and geese are only slightly susceptible. Young chicks probably are not susceptible under field conditions, though the disease may be produced artificially. Rabbits, guinea pigs and mice are slightly susceptible under laboratory conditions. The causative organism has many features in common with members of the colon-typhoid group.

5. The disease in the field usually has a mortality rate of about 30 per cent, although instances have been recorded in which the mortality was 100 per cent. It has a progressive spread from flock to flock. Within the flock, unless checked, the disease spreads until all susceptible birds are affected.

6. The means of dissemination from flock to flock is through infected soil on common range grounds; through surface washing, and through conveyors of the disease, such as man and animals. In the flock, the disease is spread through infected soil, infected food material and infected drinking water, and through eating the blood of birds dying of the disease. There is slight possibility of spread of the disease through actual contact.

7. The carrier of avian typhoid is a flock menace. Sparrows and pigeons are proven means of conveying the disease.

8. The disease has pronounced clinical symptoms, chief of which is the elevation of temperature, ænemic condition of the face, comb and wattles, and a sulphur colored discharge. The dead birds show characteristic anatomical changes, these being pronounced in the liver, spleen, kidneys and intestines. There is alteration in the blood composition, with a pronounced increase in the number of leucocytes, and decrease in erythrocytes. The hemoglobin is reduced.

9. Typhoid may be produced artificially by any method that brings virulent organisms of avian typhoid into the alimentary tract or introduces them into the blood stream.

10. Typhoid may be controlled through sanitation, isolation or destruction of suspects, safe disposition of the carcasses of birds dying of the disease, disinfection of drinking water, and the prophylactic vaccination of well birds. Vaccination produces an immunity of unknown duration, but the single inoculation should immunize the bird for the period of duration of the disease in the flock.

FOWL CHOLERA

1. Avian cholera, caused by Pasteurella avicida, is prevalent in most countries where poultry is kept. No outbreaks of avian cholera have been recorded in North Carolina during the period of these investigations.

2. The dissemination of this disease in the flock and from flock to flock is probably accomplished through the same agencies as in the case of avian typhoid.

3. There are morphological differences and differences in physiological reactions of the causative organisms of these specific diseases that differentiates them as separate and distinct germs.

4. The clinical symptoms, blood changes, and pathological anatomy of these diseases, though having much in common, are sufficiently different to establish a basis for differential diagnosis.

5. Control measures that apply to avian typhoid should be effective in checking the spread of avian cholera.

We wish to thank the following for response in sending cultures to these laboratories for further studies and other assistance they may have given: Dr. L. F. Rettger, Connecticut Exp. Station; Dr. H. G. May, Rhode Island Exp. Station; Parke-Davis Co., Detroit, Mich.; Dr. A. T. Kinsley, Kansas City, Mo.; Massachusetts Agricultural College; Dr. J. R. Beach, California Exp. Station; Bureau of Animal Industry, U. S. Dept. of Agriculture; Dr. Taylor, So. Dakota Experiment Station; Lederle, H. K. Mulford, and Jen-Sal laboratories; American Museum of Natural History; Botany Department, N. C. State College; N. C. State Laboratory of Hygiene; Lister Institute, England; Pasteur Institute, Paris; Dr. B. J. C. teHennepe, State Serum Laboratories, Rotterdam, Holland, and other workers in Belgium, Holland, and Budapest, Hungary.

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